Optimisation of fertility cryopreservation in females - from in vitro fertilization to in vitro maturation to in vitro culturing

By

Muhammad Fatum MD

A Portfolio of research submitted in partial fulfilment of the award of the degree of Doctor of Philosophy by Publication

School of Pharmacy and Biomedical Sciences
Faculty of Sciences
University of Portsmouth

Student Name- Muhammad Fatum, MD
Honorary Senior Clinical Lecturer
Head of Examination Board- MSc in Clinical Embryology
Institute of Reproductive Sciences
Nuffield's Department of Women's Health and Reproduction
Deputy Medical Director
Oxford Fertility Unit
Oxford University
Oxford

Supervisor:
Graham Mills
Room 2.25
St Michael's Blg
School of Pharmacy and Biomedical Sciences
University of Portsmouth
White Swan Road
Portsmouth, PO1 2DT
United Kingdom
T: 00 44 (0) 23 92 84 2115
E: graham.mills@port.ac.uk
W: www.port.ac.uk

Acknowledgements

Without the support of my wonderful wife, Samia and my adorable daughter Arinne, none of this would have been possible.
I have been lucky to work with a lot of talented researchers and clinicians from whom I acquired the passion for research and innovation. The studies included and the future studies are part of brilliant collaboration with these capable and inspiring individuals, research departments and hospitals in Jerusalem and Oxford. I am extremely grateful to my esteemed supervisor, Professor Graham for his invaluable advice and support.

Declaration

I declare that whilst studying for the degree of Doctor of Philosophy by Publication at the University of Portsmouth, I have not been registered for any other award at another university. Furthermore, the work undertaken for this degree has not been submitted elsewhere for any other award. The published works contained in this submission are my work and where the work of others is referenced it has been duly acknowledged in the manuscript.

Abbreviations

ART	Advanced Reproductive Technologies
ASCO	American Society of Clinical Oncology
AMP	Adenosine MonoPhasphate
EHS	Ehler Danlos Syndrome
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicular Stimulating Hormone
HCG	Human Chorionic Gonadotropin
HFEA	Human Fertilization and Embryology Authority
HTA	Human Tissue Authority
ICM	Inner Cell Mass
ICSI	Intra-Cytoplasmic Sperm Injection
IVA	In Vitro Activation
IVF	In Vitro Fertilization
IVM	In Vitro Maturation
JC-1	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi- dazolylcarbocyanine iodide
LBR	Live Birth Rate
LH	Luteinizing Hormone
LHR	Luteinizing Hormone Receptor
MEM	Minimal Essential Medium
MtDNA	Mitochondrial Deoxyribonucleic Acid
OHSS	Ovarian Hyper-Stimulation Syndrome
OTCP	Ovarian Tissue Cryo-Preservation
OUFHT	Oxford University Foundation Hospitals Trust
OXPHOS	Oxidative Phosphorylation
PCOS	Polycystic Ovarian Syndrome
PCR	Polymerase Chain Reaction

PGD	Preimplantation Genetic Diagnosis
POI	Premature Ovarian Insufficiency
P450SCC	Cytochrome P450 Side Chain Cleavage
PTEN	Phosphatase and Tensin homolog
RGD	Arginylglycylaspartic acid
RO	Reaggregated Ovaries
SCID	Severe combined immunodeficiency
SF-1	Steroidogenic Factor 1
SOP	Standard Operating Procedure
StAR	Steroid Acute Regulator
TS	Turner's Syndrome

Definitions

Implantation rate- the number of gestational sacs observed at 6 weeks of pregnancy divided by the numberof embryos transferred.

Clinical pregnancy rate- the number of cycles with gestational sac/sacs observed at 6 weeks of pregnancy divided by the number of cycles started.

Contents

Abstract

The dissertation presents my published studies investigating the optimisation of the different fertility cryopreservation modalities in females- from In vitro Fertilization to In Vitro Maturation to ovarian tissue cryopreservation and in Vitro culturing. We aim at presenting the advancement of fertility preservation options for paediatric, adolescent and reproductive aged female patients. The studies include 16 peer reviewed journal articles, two poster abstracts, three book chapters in addition to two additional articles in submission. These studies cover my academic career while working in the Hebrew University of Jerusalem and Oxford University during the last 20 years.
The dissertation includes my research on:

1. The basic reproductive physiology and infertility- this will include two book chapters in female reproductive endocrinology and pathophysiology and investigations of female infertility and a basic science study on poor ovarian responders, done in an attempt to find the pathophysiologic basis of this group of patients. We found that granulosa cells from elderly poor responders showed normal mitochondrial membrane potential and normal endocrinologic function as presented by JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) mitochondrial stain and the StAR and side chain cleavage enzyme levels. We have shown that low follicular phase oestrogen levels induced by aromatase inhibitors do not play a deleterious effect on folliculogenesis and that oestrogen levels per se are not critical for folliculogenesis in the mice ovaries. In addition, embryos created and cultured showed normal development in culture as shown by satisfactory progression to advanced embryonal forms i.e. the morula and blastocyst stages. We then suggested the safety of aromatase inhibitors utilised for superovulation in cancer patients.
2. In Vitro Fertilization (IVF) followed by embryo or eggs freeze as a well-established modality. We present several studies done, including the confirmation of random start ovulation induction protocol, the safety of egg collections in patients having haemostatic disorders which is a common condition in cancer patients, embryonal biomarkers as predictors of implantation outcomes and clinical pregnancy rates and our contribution in the study of the different

protocols utilised in frozen embryo replacement cycles, which are the mainstay of the fertility treatment after patients recover from their cancer treatment and return for embryo transfer.

3. The development of In Vitro Maturation (IVM) and its embodiment in the fertility preservation programme, as a standalone option and also as a complementary modality, together with IVF or ovarian tissue preservation options. I will present my work in this area including comparison of IVM and IVF results, using IVM and surrogacy in Ehler Danlos disease and utilisation of IVM in under responding or over responding PCOS patients. The adoption of IVM is of importance in any fertility preservation programme, as a standalone or a complementary modality.
4. Ovarian Tissue Cryopreservation as a modality in prepubertal girls or adult patients who need to proceed urgently to chemotherapy. This work culminated in setting up ovarian tissue banking in Oxford including developing protocols, policies and nationwide clinical service. I have worked on the establishment of one of the UK's first HTA/HFEA approved ovarian tissue cryopreservation programme which offers cancer patients from across the country in a hub and spoke model to have ovarian cryopreservation. A chapter in the book will be presented as well as a manuscript summarising our experience will be submitted for publication to report the first 100 patients referred to this important translational programme.
5. In vitro culture of ovarian tissue as a future fertility preservation option. We investigated the developmental staging of the different follicles in human cryopreserved ovarian tissue from patients undergoing fertility preservation. Our findings showed a variation in follicular developmental stages and their health in both cultured and non-cultured ovarian tissue. Our study reported a new classification system of follicles health in human cryopreserved ovarian tissue, based on the presence/absence of pyknosis in granulosa cells, pyknosis in oocytes and the presence or absence of shrunken cytoplasm.

These publications contributed to the adoption of well-established fertility preservation techniques as well as the innovative and experimental technique of ovarian tissue preservation that was adopted and offered to patients. My work helped to start basic science and biomedical studies to further the development of fertility preservation options in female cancer patients. Future horizons of my work would be in developing techniques of in vitro culturing of follicles, reaggregated ovaries and the biofabricated ovary.

Introduction:

Cancer patients are commonly offered chemotherapy and/or radiotherapy treatment schedules which are increasingly more effective and providing long disease remission and improving their overall survival rates and long term prognosis**.** Consequently, more cancer survivors are likely to face the late sequels of the adverse systemic effects of these anti-cancer treatments on different organs, affecting thus patients' quality of life for a long time after their recovery **(Green et al. 2009)**. In the last decades, more attention has therefore been allocated to the effects of anti-cancer treatment on gonadal function in cancer survivors **(Turan and Oktay, 2014)**. Gonadal damage might adversely affect their fertility prospects as well as their gonadal endocrine function, thus potentially affecting their growth, development as well as the long term health and general well-being **(Vassilokopoulou et al. 2016)**. The gonadotoxicity extent varies among the different chemotherapeutic agents and the different combination protocols and are generally classified as low, intermediate and high risk regimens **(Jadoul et al. 2010)**. The incidence of ovarian failure is highly dependent on the agents used, their cumulative dose and the reproductive age of the patient **(Anderson et al, 2015)**.
With the increasingly raised awareness of patients, their guardians and their clinicians to the reproductive and endocrine adverse effects of chemotherapies/irradiation therapies, there is an ever increasing need and demand to thoroughly discuss these compelling and important issues with patients and offer them satisfactory fertility preservation treatments prior to commencing on the chemotherapy/radiotherapy regimens of choice **(Wallace et al. 2014).**

Currently, there are several well established fertility cryopreservation options in females which are highly dependent on the physiologic age of the female **(Sonmezer and Oktay , 2004)**; Adult females and post menarcheal girls or post-pubertal adolescents can be offered an in vitro fertilization cycle for either embryo or oocyte cryopreservation. These patients commonly undergo ovarian stimulation in an IVF cycle and subsequently undergo oocytes retrieval prior to the initiation of chemo/radiotherapy **(Manuel et al. 2019)**. Mature eggs can then be either fertilized by partner's sperm if available or by donor sperm if patient is a single, to produce embryos to freeze or alternatively, the unfertilized eggs can be frozen by rapid vitrification when no partner is available and donor sperm is deemed ethically inappropriate or religiously unacceptable. The main advantage of this fertility preservation modality is

that it is an established technology and is in routine clinical use in most IVF units throughout the world on a daily basis **(Tulandi et al. 2008)**. The major drawback of this option is the need for almost two weeks of hormonal stimulation followed by egg collection with all inherent side effects and complications that might ensue. In addition, it is a relatively expensive treatment which adds to the difficulties these patients endure. Furthermore, only a limited number of embryos/oocytes can be created and then stored from a single or a couple of stimulation cycles **(Luke et al. 2016)**. Secondly, a less common modality and relatively further less effective option is in vitro maturation (IVM) followed by either embryo freeze or oocyte rapid vitrification **(Son et al. 2019)**. The main advantage of this technique is that patients are saved the ovarian stimulation and the related adverse effects and complications, especially OHSS. Thirdly, ovarian suppression attempted by monthly injections of GnRH analogues is occasionally prescribed prior to commencement of chemotherapy, nevertheless its efficiency in fertility preservation is still of controversial significance and its use should be largely offered in well-designed research based experimental protocols **(Blumenfeld Z. 2019)**. Fourthly, fixation of ovaries (Oopheropexy) to outside the pelvic radiotherapy field is appropriate in a sub-group of patients who would need to undergo pelvic brachytherapy **(Moawad et al., 2017).**

Ovarian tissue cryopreservation (OTCP) is an emerging fertility preservation technology that involves the harvesting of ovarian cortical biopsies, their processing into small slices followed by slow freezing or rapid vitrification. Laparoscopy is usually performed to harvest ovarian tissue either by unilateral oophorectomy or ovarian biopsies **(Ledanyi et al., 2017)**. Following patient's recovery from cancer treatment, the ovarian tissue can be thawed out and autotransplanted to the patient. Once blood supply to the autograft is successfully re-established usually within days of transplantation, and tissue proves viable and functional, folliculogenesis is resumed with possible restoration of natural fertility or endocrine ovarian activity **(Donnez et al 2010).** The tissue is most often transplanted in the pelvis in or near its natural site, such as the cortex of the contralateral ovary or the pelvic sidewall (the so called orthotopic transplantation). This has been shown to offer the potential for spontaneous pregnancy, as **ovulated** eggs are naturally released in their physiologic environment in the proximity of the fallopian tubes, obviating thus the need for IVF treatment **(Rosendal et al, 2011, Revel et al, 2011, Revel et al, 2010, Dolmans et al 2013).** Alternatively, the other often used option is undertaking heterotopic transplantation which involves autografting of ovarian tissue into non-native ectopic locations such as the arm or the abdomen. Heterotopic transplantation has certain advantages **(Oktay et al, 2004, Demeestere et al 2009)** such as being less invasive approach and an easier approach for follicular

monitoring and egg retrieval for IVF treatment; however, this clinical approach is less commonly used compared with the orthotopic approach. Orthotopic transplantation has been reported to result in more effective revascularization and less follicle loss and consequently is believed to be more effective **(Demeestere et al 2009).** The orthotopic autotransplantation is performed either by laparotomy or laparoscopy. Several ovarian cortical slices are thawed out and then either sutured to the remaining ovary or left unsutured into peritoneal windows in the pelvis and more commonly in the mesosalpinx. To date more than 100 pregnancies have been reported after autotransplantation of ovarian cortical tissue slices mainly after orthotopic but also after heterotopic reimplantation **(Donnez et al 2008)**. The published reports of worldwide centres with autografting experience indicate that it has taken 3.5 to 6.5 months on average after the autografting of the ovarian tissue until signs of endocrinologic activity (a rise in estradiol levels and a drop in FSH levels) and folliculogenesis (as observed in ultrasound scanning) were detected. Ovarian activity was observed in 93% of patients after autografting. The post-transplantation pregnancies reported thus far included natural conceptions, ovulation induction cycles and IVF cycles **(Donnez et al 2008).** The average duration of ovarian function after transplantation is 4-5 years **(Donnez et al 2008, Schmidt et al, 2005)** and is mainly dependent on the ovarian reserve before the harvesting and the absence of chemotherapy before cryopreservation. These promising results strongly suggest that ovarian tissue cryopreservation is a viable fertility preservation option that should be part of any oncofertility programmes.

As Ovarian Tissue Cryopreservation (OTCP) is the only available fertility preservation approach that can possibly be offered to premenarcheal/prepubertal girls and adult women who need to promptly and urgently start their chemo/radiotherapy and cannot undergo an IVF cycle (which entails delaying their treatment for 3-4 weeks), it is important to set up such a tissue banking service to answer the clinical need. OTCP can be performed immediately without any delay, as no hormonal stimulation is needed, and potentially thousands of follicles exist in each small cortical slice, conferring thus a quantitative advantage **(Sonmezer and Oktay, 2004).**

Several factors should be weighed up when discussing ovarian tissue cryopreservation: patient's age, patient's oncologic diagnosis, prognosis, obstetric history, fertility plans and the availability and affordability of other fertility preservation technologies. In addition, patient should be given the accurate and most up-to-date data of success rates reported so far in the pertinent literature and offered psycho-social counselling as needed. As the autografted ovarian tissue might be contaminated by micrometastases that might result in seeding of cancer cells to the cured patient, carrying thus the risk of relapse, it is important to work on in vitro culturing of the ovarian tissue to produce mature eggs for

these patients. This would obviate the need for autografting and thus minimizes the risk of relapse. This is a newly evolving area of research which would provide the upcoming breakthroughs in the field of oncofertility.

PhD plan:

In my dissertation I intend to present the works that I published in peer reviewed articles that contributed to the understanding of basic scientific and clinical biomedical knowledge in the area of oncofertility including the following topics:

1. *Introduction to the basic reproductive physiology and infertility- this will include two book chapters in female reproductive endocrinology and pathophysiology and investigations of female infertility and a basic science study on poor ovarian responders, done in an attempt to find the pathophysiologic basis of this group of patients.*
2. *IVF followed by embryo or eggs freeze as a well-established modality*
3. *The development of IVM and its embodiment in the fertility preservation programme, as a standalone option and also as a complementary modality, together with IVF or ovarian tissue preservation options*
4. *Ovarian tissue cryopreservation as a modality in prepubertal girls or adult patients who should proceed urgently to chemotherapy*
5. *In vitro culture of ovarian tissue as a future fertility preservation option.*
6. *Future Horizons*
7. *Conclusions*

1. *Introduction to the basic reproductive physiology and infertility*

The main challenge that cancer patients face once they finish their anti-cancer treatment, is the risk of compromised ovarian reserve which renders them subfertile and in extreme cases might even cause infertility **(Levine et al., 2015)**. The chemotherapeutic agents and irradiation therapy can cause this devastating damage by multiple diverse mechanisms **(Spears et al., 2019)**. Proper understanding of the reproductive endocrinology and physiology is of critical importance, providing the basis for accurate diagnosis and better targeting of the endocrinological and reproductive sequels. In order to better understand these underlying mechanisms I started my research studies to look deeper into the pathophysiological processes of folliculogenesis, oocyte and embryos development. I wrote two book chapters in the Oxford Textbook of Endocrinology and metabolism; The first chapter **(Fatum and Child, 2016, Appendix 1)** discusses the normal physiologic processes and the related reproductive anatomy. The second chapter **(Fatum and Child, 2016, Appendix 2)** pertains to the investigational workups that are utilised in order to achieve proper diagnosis of the different aetiologies of infertility, which is critical for tailoring the accurate treatment for the patients' diagnoses. In the first chapter, titled the female hormone metabolism: anatomy and physiology, I thoroughly reviewed the pertinent literature to understand the physiologic basis of folliculogenesis and the female reproductive endocrinology. As discussed in the chapter, the follicle is the basic structural and functional unit within the ovary. They are embedded in the loose connective tissue of the ovarian cortex. Only a minority of the primordial follicles are recruited for further growth and development. This recruitment induces both structural and functional changes in the follicle and especially in the granulosa cell growth and maturation. Primordial follicles then grow and develop to primary, secondary, preantral and antral follicles stages. Early stages of follicular development are FSH-independent and are mainly controlled by local intraovarian factors, i.e. Anti-Mullerian Hormone (AMH). These early stages of the follicular growth occur several preceding cycles over a time period of about 85 days prior to achieving preovulatory status. Full maturation of the follicles occurs only towards adolescence and adulthood when the female reaches her reproductive age with the maturation of the Hypothalamic-Pituitary-Ovarian axis. The follicles are then either recruited for further development and maturation or undergo atresia. Follicles are recruited by an FSH dependent process at the transition time between the preceding cycles luteal phase and the early follicular phase of the current follicular phase. A cohort of

2-6 mm antral follicles are usually recruited and start folliculogenesis until the dominant follicle is recruited and the other follicles undergo atresia. The basic endocrinologically active structure is comprised of theca cells and Granulosa cells where ovarian steroidogenesis in the follicles is regulated according to the two-cell, two-gonadotropin theory. According to this theory LH acts on the theca cells which is mediated by cyclic AMP, StAR and SF-1 leading to androgens synthesis which then diffuses to the granulosa cells. FSH acts on granulosa cells through cyclic AMP, LHR-1 and SF-1 and promotes aromatase expression and action with conversion of aromatizable androgens into estrogens. Oestradiol is the main and most important oestrogen secreted, its main functions are the development of endometrial thickness, the triggering of the midcycle LH surge and subsequent ovulation. In addition, the physiologic suppression of FSH secretion by negative oestradiol feedback is key mechanism in preventing multi follicular development and the single dominant follicle selection and development. In the second chapter, I reviewed the clinical assessment and investigations, including the history and physical examination and the biochemical and genetic investigations that are indicated whenever a disruption of the normal physiology and female endocrinologic status is suspected **(Fatum and Child, 2016, Appendix 2)**.

The main problem related to anti-cancer treatment is the depletion of the follicular pool in both ovaries leading to a spectrum of endocrinologic and/or reproductive ovarian insufficiency. If severe enough, such an exposure might lead to Premature Ovarian Insufficiency (POI). These patients may present for IVF treatment and as a result of the compromised follicular pool they might present poor ovarian response which adversely affects their chances to get pregnant. Low responders **(Gonda KJ et al., 2018)** are commonly defined as women who fulfil two out of three criteria: 1. Positive for low ovarian reserve markers 2. Previous low ovarian response with the achievement of less than 6 eggs during ovarian stimulation cycle. 3. Age older than 40 years old. In order to study the molecular and cellular reproductive mechanism of the resultant poor response, we studied the granulosa cells function in a group of older low responder IVF patients and compared them to young normal responder patients undergoing IVF cycles **(Fatum M et al., 2009, Appendix 3)**. We selected these two groups as they represented the two ends of the reproductive age with the older low responders being the study group and the young normal responders the control group. We aimed to compare the mitochondrial function in granulosa cells in both groups. We studied mitochondrial membrane potential by JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi- dazolylcarbocyanine iodide) staining as observed by confocal microscopy. In addition we performed an assay of the StAR- Steroidogenic acute regulatory protein and the cholesterol side chain cleavage cytochrome P450 (P450 scc) in granulosa cells of both groups. We

further studied the endocrinologic function within the follicles by testing Oestradiol levels in the follicular fluid retrieved during oocyte pick-up in the IVF cycles. Women in the study group were found to have a significantly higher baseline day 3 FSH levels, a lower Oestradiol levels on the day of hCG trigger, a lower number of follicles, eggs and embryos achieved. We found that the number of trypan blue-negative granulosa cells per follicle to be significantly lower in the study group compared with the control group. Western blots of nine women in the study group and four in the control group were done. As shown in Figure 1. of the article (**Fatum M et al., 2009, Appendix 3**) protein levels of the StAR and P450scc were similar, suggesting thus that these two proteins do not play a role in granulosa cells function. Figure 2 (**Fatum M et al., 2009, Appendix 3)** shows similar undisturbed granulosa cells mitochondrial membrane potential in both groups. To further validate these results we performed propidium iodide to see if there was a difference in apoptosis between both groups. As shown in Figure 3 (**Fatum M et al., 2009, Appendix 3)**, no significant difference in apoptotic cells percentages in both groups.

This study of ours was then followed by another study from our centre which aimed at examining the effect of ovarian response and age on the oxidation phosphorylation (OXPHOS) by injecting functional autologous mitochondrial concentrate from follicular fluid cells into oocytes group **(Shufaro Y et al., 2012)**. It was found that the OXPHOS function of the respiratory chain in mitochondria is unaffected by ovarian response and age. In addition the mtDNA was intact in all samples. These results are in keeping with our results concerning the normal functionality in granulosa cells regardless of the ovarian reserve and the age.

Both studies were done in an attempt to establish the feasibility and safety of using mitochondria from granulosa cells to rejuvenate eggs in low responders or elderly patients, by injecting mitochondria from granulosa cells of the same patients, thus obviating the need and the potential complications of injecting heterologous mitochondria from younger fertile women. The main drawback of our study of our study was that it did not study the endocrinologic and mitochondrial functioning of granulosa cells in the non-responding follicles which constitutes an important component of follicular population, where there may still be a possible defect in the steroidogenesis with resultant hypoestrogenic environment.

In order to simulate this hypoestogenic environment and study the importance of proper secretion of estrogen during folliculogenesis, we performed an animal model study, and injected Arimidex (Anastrozole), an aromatase inhibitor to C57Black female mice in order to induce low estrogen levels

(Fatum et al., 2006, Appendix 4). We examined whether hypoestrogenic microenvironment would have an impact on the folliculogenesis, the competence and maturation of the eggs and in vivo ovulation. In addition we studied the effects of Arimidex administration on the in vitro development of embryos into morulae, blastocysts and hatching blastocysts in culture. This study was also done to study the safety and the effects of using Anastrozole for ovulation induction in poor responders, as a number of publications reported the viability and efficacy of using aromatase inhibitors in ovulation induction especially in poor responders and in estrogen-receptor positive breast cancer patients who might benefit from reducing the estrogen levels during ovarian stimulation in IVF cycle done to freeze eggs or embryos as a fertility preservation option. Our study was unique as it studied the effects of Anastrozole on the folliculogenesis and the subsequent development of embryos in culture. We confirmed the efficacy of Anastrozole injection into the mice in reducing estradiol levels in the circulation. A significant reduction in oestradiol levels was demonstrated in the Arimidex group in comparison to the control group. Nevertheless, the two groups did not differ by the total number of developing follicles nor by the distribution for antral and pre-antral follicles **Table 1. and Figure 1 (Fatum et al., 2006, Appendix 4).** In addition, and despite the low Oestrogen levels, the total numbers of embryos resulting from mating these mice were similar in the control and study groups, as well as the embryonic development of the embryos into the morula, blastocyst and hatching blastocysts stage. This study provided evidence that low follicular phase oestrogen levels do not play a deleterious effect on folliculogenesis and that oestrogen levels per se are not critical for folliculogenesis in the mice ovaries. In addition, embryos created and cultured showed normal development in culture as shown by satisfactory progression to advanced embryonal forms i.e. the morula and blastocyst stages. This study showed that aromatase inhibitors could possibly be safely used as part of the ovarian superovulation in IVF cycles in general and potentially in hormone-receptor positive cancer patients, where clinicians would prefer patients are kept with low oestrogen levels, due to potentially deleterious effects of high levels of oestrogen. Several studies and other reports have demonstrated the effective use of aromatase inhibitors in ovarian stimulation in IVF patients and in cancer patients **(Ben-Haroush et al., 2019, Pereira et al., 2016).** Nowadays, aromatase inhibitors are widely used in IVF and fertility clinics and are considered to be effective adjunct therapies in combination with gonadotropins injections **(Balen et al., 2016).**

We then did a review of the pertinent literature to assess the safety of using aromatase inhibitors for superovulation and study whether congenital malformations rate was increased in these patients. Early

small retrospective studies suggested a possible increased congenital cardiac and bone fetal malformation rate in patients who were using aromatase inhibitors for ovarian stimulation **(Biljan et al., 2005).** Our review of the literature and analysis of the different studies (for a more detailed description of the studies included, see (**Fatum et al., 2006, Appendix 5**), had shown the relative safety of using these agents for ovulation induction. These studies including animal studies and clinical human reports indicate that aromatase inhibitors are relatively safe for usage in ovulation induction in terms of folliculogenesis, in vitro embryo development and relative safety vis-a-vis teratogenic effects. As these medications are used during IVF cycles done for fertility preservation before the commencement of chemotherapy and irradiation and in these circumstances embryo transfer is done years after ovarian stimulation, there seems to be no safety issues. To the contrary, in the case of hormone-receptor positive tumours, the low oestrogen levels in the circulation may be advantageous in these patients. Several studies in the literature followed, which validated our results and reinforced the utilisation of these medications in ovarian stimulation **(Moini et al., 2019, Legro et al., 2014).**

2. In Vitro Fertilization followed by embryo or eggs freeze as a well-established modality.

As IVF followed by embryo or eggs freeze, is the main fertility preservation option, I will present the studies done, including the random start ovulation induction protocol, the safety of egg collections in patients having haemostatic disorders which is a common condition in cancer patients, embryonal biomarkers as predictors of implantation outcomes and clinical pregnancy rates and our contribution in the study of the different protocols utilised in frozen embryo replacement cycles, which are the mainstay of the fertility treatment after patients recover from their cancer treatment and return for embryo transfer.

Fertility preservation in cancer patients prior to gonadotoxic anti-cancer treatment is a rising clinical service and has been an ever-growing discipline, that recently was even granted a specialised nomenclature - oncofertility **(Jeruss and Woodruff, et al., 2009; Salama et al., 2019)**. The numbers of publications on this risen topic has increased steeply in the last few years due to the new breakthroughs in the field and the constantly evolving state-of -the-art practices in the different fertility preservation modalities in female oncologic patients **(Ataman et al., 2018)**. Oncologic patients are increasingly referred for fertility preservation consultation prior to the commencement of chemotherapeutic/irradiation treatment. As the patients' awareness to the availabilities of the fertility preservation options increases and as the general practitioners and oncologic physicians knowledge gap of the different oncofertility modalities is being increasingly bridged, fertility preservation service is becoming an integral part of the clinical care of cancer patients **(Jeruss and Woodruff, et al., 2009)**. Upon their referral, patients are thoroughly assessed to establish the need and the indication for fertility preservation. This assessment takes into account the diagnosis, chemotherapeutic agents and the irradiation scatter that is planned, their gonadotoxic potential, the urgency with which the fertility preservation treatment should be offered, age of the patient and the five years survival of these patients **(Michaeli, et al., 2012)**. There are several algorithms for the different cancer diagnoses and the relative gonadotoxic potential associated with these cancers and their recommended treatment protocol and whether fertility preservation is indicated for these patients. In addition, similar algorithms and databases provide estimates of the gonadotoxic effects of different chemotherapeutic agents and irradiation protocols to aid clinicians in providing accurate recommendations to cancer patients. ASCO

guidelines provide evidence based recommendations for the vast majority of cancers and anti-cancer treatment protocols (**Oktay, et al., 2018, Knight, et al., 2015**).

The next step in the decision making tree is to decide on the fertility preservation modality to be offered to patients. In adolescents and pubertal patients, an IVF cycle provides the first best option in intermediate-high risk patients. This option gives a good reliable fertility preservation in the form of egg freezing or embryo freeze. All IVF units can possibly offer these patients a cycle of ovarian stimulation and egg collection. This is followed by either fertilisation of these eggs by partner's or donor sperm and embryonal freeze, or by vitrification of eggs if there was no partner or if the use of donor sperm is not considered by patient or her guardian. Some patients might opt to do both options with half of the patients eggs being vitrified and the remaining half fertilized with sperm followed with embryo freeze. In premenarcheal girls and in pubertal/adolescent patients who must start anti-cancer treatment urgently, ovarian tissue preservation is offered. This well be further discussed in chapter 5 (**Fatum and McVeigh, 2013, Appendix 6**).

Offering IVF cycle for fertility preservation can be accompanied by various challenges that the clinician should take into account and would need to thoroughly discuss them with patients before an informed consent is given. Patients should be made aware that the treatment cycle might cause 2-4 weeks delay in the start of chemotherapy or radiotherapy depending on the day of the cycle the patient is and whether complications i.e. Ovarian Hyperstimulation Syndrome (OHSS) and its sequels take place, imposing further delay. Ideally, the ovarian stimulation should be commenced at the early follicular stage so that a higher number of antral follicles could be recruited resulting in a higher harvest of eggs. The number of eggs retrieved is critical for fertility preservation, as their number is finite and thus conferring a limited extent for fertility preservation. Henceforth, the aim is to safely get the highest possible number of eggs retrieved and stored giving a satisfactory fertility preservation potential **(Mizrachi et al., 2020).**

An IVF cycle for fertility preservation comprises of several components- ovarian stimulation to recruit growing follicles followed by oocyte pick up and storage of eggs or embryos, depending on the patient marital status and whether she would consider the option using donor sperm to fertilise the eggs (**Mizrachi, et al., 2020**). There are two major possible stimulation protocols that are utilised for ovarian stimulation- the short and the long protocols (**Manuel, et al, 2019, Lambalk, et al, 2017**). The long protocol is usually started on day 21 of the cycle for inducing down regulation of the pituitary gland, followed by daily injections of gonadotropins for follicular stimulation. This protocol may take 6-8

weeks to accomplish, which might prove too long for cancer patients who need to start their ovarian stimulation within 2-4 weeks. Due to this prolonged course, the short protocol was offered in cancer patients in an attempt to reduce the time scale of ovarian stimulation and allow patients to get their anticancer treatment as soon as possible. Short protocol includes the use of GnRH agonists or antagonists in the early days of the cycle in parallel to the administration of gonadotropin stimulation. Such a cycle may take 3-4 weeks if the patient is lucky to be near the beginning of her menstrual cycle **(Manuel, et al, 2019, Lamballk, et al, 2017)**. However, the majority of patients might present in late follicular phase or the periovulatory or the luteal phase where they have to wait till the beginning of the next menstrual cycle to be able to start the stimulation in the early follicular phase. This might be unacceptable to those patients who need to start chemotherapy in a duly time. A third option has been suggested in the literature in the context of fertility preservation- the random start protocol **(Nayak, et al., 2011)**. In this protocol- regardless of the menstrual cycle phase and especially in the luteal phase, patients are started on GnRH antagonists for three days before the administration of gonadotropin stimulation. Its main advantage is that no further waiting is required before starting the stimulatory protocol **(Cakmak, et al., 2015)**. The classic theory of folliculogenesis and ovarian stimulation advocates the early follicular phase stimulation as the ideal timing of stimulation as more follicles are possibly recruited in that phase. As the stimulation is delayed to a later stage of the follicular phase, the number of follicles recruited drops significantly **(Hsueh, et al., 2015)**. All the more so, luteal phase stimulation has been perceived as a counter physiologic regimen. Concerns have arisen concerning the number of eggs that are harvested using the random start protocol. For answering this question, we have run a retrospective study to compare the outcome of random start protocol to the usual stimulatory protocols to assess the efficacy of the random start protocol **(Muteshi, et al., 2018 Appendix 7)**. The study included 137 cancer patients that were referred for fertility preservation and underwent ovarian superovulation for gamete/embryo freeze between February 2003 and June 2016. The random start protocol has been offered at any point after the fifth menstrual day. It involved the administration of Cetrorelix 250 micrograms (Cetrotide, Serono Pharmaceuticals Ltd, Feltham, UK) for a total of three days before ovarian stimulation was commenced on the fourth day. The Cetrorelix was continued until the day of the HCG or Buserelin administration. A multivariable logistic regression analysis was then performed. 127 patients were treated using the antagonist protocol (103 (81%) in the conventional early follicular start group and 24 (19%) in the random start group). As the results show, both the study and control groups were similar in their epidemiological parameters as presented in table 1 **(Muteshi, et al., 2018 Appendix 7)**. We have found similar outcomes using both antagonist

protocols: there was no significant difference in the number of eggs retrieved, eggs fertilised and embryos stored between both groups. Consequently, we could conclude that the random start protocol is equally effective whilst saving patients unnecessary waiting time to start the treatment with the random start protocol. This study is among a limited number of studies in the literature on this topic **(Nayak, et al., 2011, Ortega, et al., 2018)** and was quoted in the 2019 ESHRE guidelines of ovarian stimulation **(eshre stimulation guidelines)**. Nowadays the random start is increasingly becoming the treatment of choice in cancer patients requiring IVF treatment for fertility preservation.

Our aforementioned studies, discussed in the previous chapter, with regards to the utilisation and safety of aromatase inhibitors in ovarian stimulation, contributed to the introduction and adoption of these medications in ovarian stimulation protocols in cancer patients and in ovulation induction in fertility patients **(Chapter 2, Fatum, et al., Appendix 2).** We have shown the relative safety of using the Anastrozole in folliculogenesis, oocyte maturation and the embryonic development in culture in mice. In addition, we analysed the different studies on the safety of Letrozole in pregnancy. Henceforth, we published an opinion article supporting the usage of aromatase inhibitors in cancer patients having estrogen receptors positive tumours **(Appendix 5)**. With time Aromatase inhibitors have become an integral part in ovarian stimulation protocols in fertility preservation cycles **(Moini, et al., 2019, Azim, et al., 2008)**.

When discussing the fertility preservation procedure to patients, it is important to keep patients well informed of the true fertility preservation potential that the frozen eggs or embryos confer. This is of critical importance in their informed consent and reassurance, so that they have realistic expectations of the fertility preservation potential. In addition this is crucial also for the clinician to decide whether more than one IVF cycle might be needed. We did a study that aimed to examine the different morphokinetic characteristics of the blastocysts as predictors of implantation potential and ongoing pregnancy rates **(Subira, et al., 2016, Appendix 8, Subira, et al., 2014**). We designed a retrospective cohort study of 1084 fresh elective single blastocyst transfers. We assessed the following grading parameters in the different blastocysts transferred: blastocyst expansion, the inner cell mass (ICM) grade and the trophectoderm grades, according to Gardner's blastocyst grading system **(Gardner and Schoolcraft, 2019)**. Our primary outcome was the live birth rate (LBR) and the secondary outcomes were implantation rates and clinical pregnancy rates and early pregnancy loss rates. Using definitive multivariable regression analysis, we found that the ICM and blastocyst expansion were associated with

LBR. We found that when the ICM grade dropped from grade A to C the likelihood of LBR was significantly reduced by 55%. Similar results were also shown for the clinical pregnancy rates predictors. In addition, we found that early pregnancy loss rates in embryos with ICM grade C were more significantly increased compared to those with grade A and grade B (38.0% vs 15.95% vs 17.17%, p = 0.002). The transfer of a single embryo with a high ICM grade reduces the likelihood of early pregnancy loss and increases the likelihood of live birth. Other studies in the literature have shown conflicting findings and some reports suggested that the trophectoderm grade is a better predictor **(Thompson, et al., 2013)**. As these results may well show the variability of the findings in the different studies, this might reflect different laboratories protocols utilised and the different clinical practices being applied in each centre. Therefore, we suggest that each fertility preservation programme advise their patients of their own results and figures with regard to the programme's predictors of clinical pregnancy rates and LBR, as currently there is no clear consensus as to which predictor is better as an indicator (**Thompson, et al., 2013**). We believe that providing this information is crucial to coordinate patients expectations of the embryos stored.

Cancer patients, especially those with haematologic diagnoses may present with conditions that increase their bleeding predisposition, i.e. thrombocytopenia or prolonged bleeding time either due to their oncologic disease (for instance, leukaemias and lymphomas) or as an adverse effect of the chemotherapy they might have been exposed to prior to their referral. One of the main clinical concerns in these patients is the possible haemorrhagic complications that might ensue during or after oocyte pick up, especially due to the hypervascularity of the overstimulated ovaries. In order to assess the safety of performing egg collection procedure in these patients- we did a retrospective cohort study on all patients who have had a history of bleeding tendency and who underwent egg collection after appropriate preoperative preparation and correction of the haemostatic abnormalities (**Fatum, et al., 2007, Appendix 9**). We found eight patients who were found to have a haemostatic disorder including thrombocytopenia due to ITP, VW factor deficiency or factor VII or XI deficiencies as described by **Table 1**. **(Fatum, et al., 2007, Appendix 9).** We had demonstrated the relative safety of oocyte pick up in all of these patients, provided the pre-procedure bleeding tendency is timely diagnosed and patients are properly prepared preoperatively to restore their haemostatic competence by disorder specific treatment. This was an important report and among the first reports in the literature to demonstrate the feasibility and relative safety of offering oocyte pick up in patients found to have haemostatic defect **(Fatum, et al., 2007, Appendix 9)**.

Once an oncologic patient has finished her anti-cancer treatment and fully recovered from her disease, a debatable waiting period of time, commonly one-two years period of follow up is recommended before transferring embryos and attempting pregnancy. This is of importance in order to make sure patients have been in long standing remission and further reduce the risk of relapse while being pregnant (**Lawrenz, et al., 2011**). This could be further delayed for longer periods if additional adjuvant treatment is needed, i.e. in breast cancer patients where longer treatment with Tamoxifen is commonly initiated for several years after the completion of the chemotherapy.

The treatment that follows includes mainly a hormonal treatment to artificially prepare the endometrial lining towards egg/embryo thaw out and embryo transfer. These so called medicated frozen embryo replacement protocols are of importance, as these patients may have endured premature ovarian insufficiency and might not have menstrual cycles or their natural cycles are not ovulatory. In these cases, exogenous hormones should be administered to prepare the lining. The vast majority of women would respond well to the administration of oestrogens to thicken the endometrial lining, followed by the addition of progesterone pessaries for luteal support. This treatment is continued till the 8th gestational week. In the reproductive medical literature, this could be done in either a long protocol using GnRH agonists starting in the mid luteal phase or in a short protocol using GnRH antagonist, to prevent spontaneous folliculogenesis and premature ovulation whilst being on oral Oestrogen medications to stimulate endometrial thickening **(Glujovsky, et al., 2010).** Other groups have also shown the safety and effectiveness of not giving either agonists nor antagonists for the inhibition of folliculogenesis and ovulation **(Simon, et al., 1998, Ghobara, et al., 2017)**.

We performed a prospective randomised controlled study to evaluate a simplified approach of artificial endometrial preparation, comparing two doses of oral Oestradiol tablets combined with a novel vaginal natural progesterone pessaries (100 mg Endometrin). Twenty-nine patients were recruited in the study and divided randomly into two groups. The first group received oral Oestradiol tablets 4 mg/day from the first day of menstruation and the second group were put on 6 mg/day. Following an overall 12 days of oestradiol priming and after with an endometrial thickness of > = 8 mm, Endometrin vaginal tablets 100 mg were added twice a day for a total of 10 days. On day 21st an endometrial biopsy was taken to evaluate the endometrial secretory changes. We have found appropriate changes in estradiol, progesterone and endometrial response **(see Figures 1-3, in Appendix 10a , Lewin, et al., 2002, Appendix 10a)**. As expected the oestradiol levels were significantly higher in the higher

Oestradiol dose, however there was no significant difference in the serum progesterone levels or the endometrial thickness in both groups. The histopathological evaluation of the endometrium on day 21, revealed adequate secretory endometrium in both groups. These results demonstrated that an appropriate endometrial thickness can be achieved using a fixed low dose oral oestradiol (as low as 4 mg/day) and that adequate late-secretory endometrial transformation can be achieved by the administration of low-dose vaginal natural progesterone. This is in line with other reports in the literature showing different progesterone medications and different methods of administration to be equi-effective in giving adequate secretory transformation and pregnancy support. This study is of special importance in cancer patients, where lower doses of hormonal treatment would be preferable in patients who are known to have hormone-receptors positive tumours.

We then did another retrospective observational cohort case study, in an attempt to study the endometrial responsiveness in patients having a history of thin unresponsive endometrium despite the administration of oestradiol in usual doses. We retrospectively analysed and studied the different artificial endometrial stimulation protocols that were used in an attempt to get the endometrial lining to adequately respond and reach an acceptable thickness. Thirteen women were included who underwent 99 cycles of artificial endometrium stimulation. This group of women had undergone various and diverse empirical treatments with different combinations and doses of Oestradiol In combination with Sildenafil and Aspirin. The different treatment modalities and combinations as shown in table 2 (**Shufaro, et al., 2008, Appendix 10b)** had various success rates in achieving adequate endometrial response and pregnancy rates. As shown in table 2 (**Shufaro, et al., 2008, Appendix 10b**), no treatment was found to be more beneficial than the other. Out of 99 treatment cycles, in only 22 cycles an adequate endometrial response was achieved; however the pregnancy rates were low with poor reproductive outcomes even if endometrial thickening or implantation was achieved (8 early miscarriages, two terminations due to malformations and one live birth). This poor prognosis and the failure of the different treatment protocols to improve the reproductive outcome is in accordance with other published data **(Bu et al., 2016, Mahajan and Sharma, 2016).**

Therefore, this challenge continues to be a hurdle to these patients who are dependent on responsive lining for their frozen embryo replacement treatment.

3. *The development of IVM and its embodiment in the fertility preservation programme.*

Oxford Fertility Unit is the first unit in the UK to offer in vitro maturation (IVM) treatment, which is offered mainly to PCOS patients; however, it is also a viable option to produce eggs for the sake of fertility preservation. I will present my work in this area including comparison of IVM and IVF results, using IVM and surrogacy in Ehler Danlos disease. The adoption of IVM is of importance in any fertility preservation programme, as a standalone option but also as a complementary modality, together with IVF or ovarian tissue preservation options.

IVM is a technology that has been utilised in the last few years as an alternative to In Vitro Fertilization (IVF) which reduces the risks of ovarian hyperstimulation syndrome (OHSS)**(Yang and Chian, 2017)**. This technique is based on immature oocyte pick up without ovarian stimulation, followed by in vitro maturation of the harvested eggs in the lab **(Chian, et al., 1999)**. As a result, patients are not exposed to high levels of ovarian stimulatory drugs, unlike the common IVF treatment cycles, and consequently the Oestradiol levels remain low and therefore there is no risk for OHSS. This technology is mainly offered to patients having polycystic ovaries or those who are at high risk of OHSS. In addition, some reproductive medicine centres utilise this technique for women who should be kept with low oestradiol levels during their fertility treatment, i.e. patients with estrogen-receptor positive tumours. In these patients, keeping the estrogen levels low is perceived as essential to their safety as clinicians are increasingly concerned with the possible deleterious effects of the high estradiol levels in these estrogen receptor positive tumors **(Grynberg, et al., 2016)**. The IVM programme at the Oxford fertility unit was the first and only fertility unit offering this treatment for fertility patients. The development and maintenance of the IVM programme in the unit was instrumental in adding this modality into the ovarian tissue cryopreservation service that was later introduced in Oxford. This enabled the introduction of in vivo and ex-vivo oocyte retrieval for IVM as part of the ovarian tissue cryopreservation and as a separate independent modality for patients that need egg collection at short notice as they need to proceed with chemotherapy or radiation therapy within days of their diagnosis. In order to keep the programme running and available for cancer patients we did several studies to establish its efficacy, safety profile and widen the indications.

In order to compare the outcome of IVM and routine IVF for women diagnosed with polycystic ovaries (PCO), we did a retrospective case control study to compare the live birth rates per cycle and the OHSS rates in both groups. The study included ninety-seven patients undergoing IVM treatment compared with ninety seven patients undergoing IVF/ICSI, all having ultrasonographic evidence of PCO and matched for age. In the IVM cycles hCG was administered 35-40 hours before oocyte retrieval. Oocytes were then matured in vitro for 24-48 hours before insemination was done by ICSI. Endometrial priming with additional oestradiol and progesterone was commenced after the egg collection and one to two good quality embryos were then transferred on days 2-5 of development. For the IVF/ICSI cycles, the standard long protocol was used for the control group. As demonstrated in **Table 2** in **(Gremeau, et al., 2012, Appendix 11)** we managed to achieve 65% in vitro maturation rate of eggs in the IVM group. The implantation rates were significantly higher in the the control IVF group (19.4% vs. 12.9%) and similarly the clinical pregnancy rates (50.5% vs. 19.6%) and the live birth rate (44.3% vs. 16.5%) were higher in the control group than the IVM. However, the OHSS rate was observed to be significantly higher in the IVF/ICSI control group (8.2% vs. 0%).

We concluded that in PCO patients who are eligible for IVM treatment, the technique offers them a reasonable chance of conceiving while obviating the need of gonadotropins stimulation and eliminating the risk of OHSS. IVM has thus been shown to be a safer and simpler alternative to IVF/ICSI treatment in PCO patients. This study has shown IVM as a viable option fertility treatment option that could possibly be offered with the appropriate clinical set up and indications for fertility preservation. This study is in keeping with other studies in the literature showing the success rates of IVM cycles in PCO patients to be almost 50% of IVF/ICSI cycles **(Child, et al., 2002)**.

We then reported the utilisation of IVM in a patient of vascular type Ehler Danlos Syndrome (EDS) who experienced postoperative bleeding from aneurysmatic vessel after IVF cycle **(Bergeron, et al., 2014 Appendix 12)**. Patients with this disorder are prone to spontaneous arterial and visceral ruptures. The occurrence of these life threatening complications is increased in pregnancy**.** This was attributed to the high estrogenic levels associated with ovarian hyperstimulation in the previous IVF cycle. The patient in this case report was a 33 years old, suffering from EDS with a history of recurrent ruptures of arterial aneurysms and recently she was diagnosed with a ruptured splenic arterial aneurysm. The patient had then undergone several natural IVF/ICSI cycles in order to avoid high oestrogen levels possibly linked to the previous complications. She then underwent two uncomplicated

IVM cycles with PGD in our clinic in an attempt to avoid high Oestrogen levels and increase the number of eggs retrieved. The patient underwent embryo transfer of a normal blastocyst; however, the PGD cycle was unsuccessful, though the safety of IVM in this vascular-type EDS patient was proven as a matter of principle **(Bergeron, et al., 2014, Appendix 12)**.

We then had a study that offered to widen the range of IVM indications to PCOS patients who over respond or under respond to ovarian stimulation during IVF treatment (**Fatum, et al., 2020 Appendix 13, Fatum, et al., 2013, Appendix 14**). This was a case series study that included two groups of patients- the first group comprised of PCOS patients undergoing IVF treatment, who over responded to the ovarian stimulation drugs with high levels of oestradiol and increased risk of OHSS, whilst follicles were still small and medium sized and needed longer stimulation period to mature. However, as further stimulation would be risky, as high estrogen levels would continue to increase and thus increasing the risk of OHSS. In these cases, either cycle cancellation or coasting are reasonable options, they can possibly compromise the outcome of the cycle. We showed that conversion to a rescue IVM cycle with egg collection in these patients followed by treatment of the eggs with IVM media would be a safe option as the trigger is given while the oestrogen levels are kept at their initial high levels with no further elevation in their levels, reducing thus the associated risk of OHSS. A total of 58 out of 68 oocytes retrieved were mature or matured in vitro. There were 26 cleaving embryos obtained. Two patients had live births and one suffered from a miscarriage. In the second study group, a rescue IVM was offered to PCOS patients who responded poorly to the highest doses of hormonal stimulation. In these two patients, a total of 22 out of 26 oocytes retrieved were mature or matured in vitro using IVM media. There were 13 cleaving embryos obtained. One patient had a live birth, whilst the other suffered a miscarriage. This study was accepted for publication this year and it adds to the diversity of treatment options for these patients, **see Table 1 and Figure 1 (Fatum, et al., 2020 Appendix 13).** We are aware of another study that suggested the feasibility of rescue IVM as a viable option **(Coskun, et al., 1998, Jaroudi, et al., 1999).**

Introducing the IVM programme and validating the efficacy of in vitro maturation of immature oocytes has been instrumental in its translational application in fertility preservation.

Upon setting up the ovarian tissue cryopreservation programme in Oxford as will be demonstrated in the next chapter,, we could reliably offer this treatment modality as part of the fertility preservation repertoire. We then presented our experience that was consequently published in two poster abstracts in AAGL 2014 and ESHRE meeting in 2015, **(Fatum, et al., 2014 Appendix 15, Fatum**

et al., 2015 Appendix 16) . We reported a combined approach in which ovarian tissue cryopreservation is offered to appropriate patients together with either in situ or ex vivo oocyte collection followed by IVM. We were among the first fertility preservation programmes that embodied the IVM as a complementary option for the ovarian tissue preservation. This experience will be the basis for an either independent article or as a subsection of an article that will summarize our experience in introducing ovarian tissue cryopreservation clinical service (see next chapter for more details).

While IVM has been reported as an independent modality of fertility preservation by different other groups **(Kedem, et al., 2018)**. However, as we have shown before **(Bergeron, et al., 2014 Appendix 12)**- the pregnancy outcomes of IVM embryos is much less **(about 50%)** than the IVF cycles, and henceforth, we believe that in the context of fertility preservation, and whenever time to start chemotherapy allows, an IVF cycle with the intention of freezing eggs or embryos, should be the first choice procedure. Using IVM as the primary fertility preservation technique, should be kept to patients who need to start chemotherapy within days, where further delay would lead to aggravation of the patients' prognosis. Grynberg et al. have offered luteal or even several cycles of IVM with back-to-back oocyte pick up to allow pooling of a higher number of immature eggs to increase the efficacy of the IVM as a fertility preservation in these patients **(Grynberg, et al., 2016)**.

4. *Ovarian Tissue Cryopreservation*

In the last few years, I have worked on the establishment of one of the UK's first HTA/HFEA approved ovarian tissue cryopreservation programme which offers cancer patients from across the country to have ovarian cryopreservation. A chapter in the book will be presented as well as a manuscript summarising our experience will be submitted for publication (myself as a first author) to report the first 100 patients referred to this important translational programme.

It is estimated that almost two thousand new cancer diagnoses are made each year within the UK with almost half of them being in premenarcheal paediatric girls. These patients until recently did not have a fertility preservation treatment due to the lack of effective treatment. Ovarian tissue preservation was offered as a promising emerging modality although there was no evidence of its effectiveness. Therefore, the NICE guidelines continued to recommend ovarian cortical tissue cryopreservation as an experimental medicine that should be done only in a well-designed research framework **(NICE guidelines link**). Groups in Edinburgh and Leeds held a research based protocol and programme for the cryopreservation of ovarian tissue for these patients **(Wallace, et al., 2014)**. However, there was no appropriate HTA and HFEA licences to make clinical use of these stored tissue and to permit their autografting back to patients once they were cured and entered their reproductive phase of their adult lives. Up till the late nineties, no pregnancies were achieved despite the harvesting of ovarian tissue in paediatric oncologic patients for fertility preservation. As a result, we wrote an opinion letter and recommended against the routine utilisation of ovarian tissue cryopreservation in oncologic patients, as there was no supportive evidence to its efficacy and the benefit /risk ratio **(Schenker and Fatum, 2004, Appendix 17)**. However, after the first pregnancies started to appear and to be increasingly reported in the literature, the status of this innovative technology changed from research area to experimental medical field. As I worked with one of the pioneering groups in ovarian tissue cryopreservation in the world who achieved live births from the transplantation of the stored ovarian tissue **(Meirow et al., 2005; Revel, et al., 2011)**, I was then offered a collaborative fellowship by Oxford University as a senior research fellow to set up a clinical Oxford Ovarian Tissue Cryopreservation Programme for cancer patients.

This work commenced in late 2008 and culminated in the achievement of both HFEA and HTA licensing of the Oxford ovarian tissue cryopreservation programme in 2013. We achieved both authorities licences in order to be able to utilise the ovarian tissue itself which is mainly in the regulatory area of the HTA, and the HFEA licence to enable the programme to process oocytes ie IVM of immature eggs. This feat included intensive efforts to finalise Laboratory SOPs and modify them as necessary, to accommodate for the European Union standards and regulations, then validating the protocols on pig ovaries, testing and validating the clean room standards of the surgical theatres at the John Radcliffe Hospital and finally getting the institutional permissions from the Oxford University Foundation Trust.

I published our initial experience in setting up the ovarian biobanking programme in a book chapter in reproductive surgery in assisted reproduction which included our modified protocols of tissue processing, freezing and thawing out (**Fatum and McVeigh, 2015, Appendix 6**). To our knowledge, our service is among the first if not the first to abide by the new European regulations for tissue cryopreservation and by publishing it, we hope to facilitate the licensing of other ovarian tissue cryopreservation programmes within the European Union to benefit from these uptodate SOPs. Oxford ovarian tissue biobanking programme has offered several unique contributions to the field of ovarian tissue cryopreservation: Firstly, The programme introduced a combined fertility preservation option including ovarian tissue cryopreservation and in situ and ex vivo oocyte pick up followed by IVM of these immature oocytes. The programme has offered the first 20 patients the option of undergoing IVM in addition to the normal ovarian tissue harvesting. We have shown as a proof of principle that this option is feasible and we have achieved immature eggs from patients as young as two years old with a reasonable maturation rate after IVM treatment (**see previous chapter and Fatum, et al., 2015 Appendix 16**). Secondly, as this service was pioneering at the national level, with it being the first to get HTA and HFEA licensing, we directly built up schemes to offer it for all patients across the UK. We have built up two schemes- first one was the referral of patients to OUFHT as a tertiary centre, where patients underwent the ovarian tissue harvesting and the processing of the tissue, followed by the slow freeze. The second scheme was through third party contracts where patients were offered to undergo the harvesting at their local hospital, followed by transporting the ovarian tissue with ice to the Oxford Ovarian tissue biobank. This scheme had the advantage of keeping the patients in their local hospital so that they could start their cancer treatment as soon as they recovered from the surgery.

Thirdly, after the successful introduction of the ovarian tissue cryopreservation, the same laboratory infrastructure and facilities were utilised to introduce a testicular tissue cryopreservation programme.

Fourthly, establishing the clinical programme has been instrumental in the launching of different research studies aiming at improving this innovative technology. This will be further discussed in chapter 6, concerning current research projects and future ones.

I have written a manuscript that summarises our experience from the first one hundred referrals for ovarian tissue preservation. The manuscript will be submitted to a reproductive medicine journal with me as the first author. Further details are presented in **Appendix 18** and A**ppendix 6**.

Being one of the first pioneering units in the UK and in the World, is an opportunity to take active part in the discussions of new indications for ovarian tissue preservation. Some of the new indications are unique benign or genetic conditions that may be a challenge to the oncofertility professionals, as there are no clear guidelines with regard to the fertility preservation indication and methods offered. Last year we published an article which reviewed the pertinent literature concerning fertility preservation options in girls with Turner syndrome. We discussed the different clinical, psychological and ethical dilemmas faced when facing this clinical entity in these young TS girls. We then built up a flow chart algorithm to help clinicians in their decision making of the different fertility preservation modalities for both pubertal and pre-pubertal girls with Turner syndrome, as appears in figure 1 in **(Jeve, et al., 2019 Appendix 19)**.

5. *In vitro culture of ovarian tissue as a future fertility preservation option*

In vitro culture of ovarian tissue is the forefront of fertility preservation studies, in patients having ovarian tissue preservation. A first publication from last year is presented together with future implications.

From the outset, and in parallel to the licensing of the clinical ovarian tissue cryopreservation programme, I have worked to get basic science research projects to further develop the technology and try to answer the challenges it poses. One of the main challenges in the application of ovarian tissue cryopreservation, is the risk of micrometastasis seeding of the primary tumour with the autografting of the ovarian tissue slices, once the oncologic patient is interested in fertility. Other than histopathologic, immunohistochemical staining, molecular tests ie PCR, to rule out the presence of the tumour cells in a sample of the ovarian tissue, there are not currently any viable options to further reduce or even eliminate the risk of tumour metastases. This is of special import in haematologic disorders i.e. leukaemia as per definition the cancer cells are in the blood vessels everywhere throughout the body including the normal ovarian tissue. In order to meet this clinical need and try to eliminate this risk, I have set out a research project for in vitro culture of ovarian tissue and follicles. The aim of such research is to advance the in vitro folliculogenesis in an effort to get in vitro matured eggs without the need of autotransplantation of ovarian tissue and thus preventing the risk of micrometastasis.

A collaboration was initiated with two labs in the Oxford University - the tissue bioengineering and bioprocessing department and the ovarian cryopreservation laboratory. The original ethical approval is enclosed **in Appendix 20** which includes the collaboration with the department of bioengineering and a further modification to include the collaboration with ovarian cryopreservation laboratory was applied and appears in **Appendix 21** which sheds light on the prospects of research projects planned.

We have recently achieved our first publication in this area from last year. In the current study **(Walker et al, 2019, Appendix 22)**, we investigated the developmental staging of the different follicles in human cryopreserved ovarian tissue from patients undergoing fertility preservation. Our findings showed a variation in follicular developmental stages and their health in both cultured and non-cultured ovarian tissue. Interpatient variation was also documented in the pre-culture samples both in the rates of healthy follicles and the different phases of growing follicles. A variation in follicular health and growth was also documented after the culturing of the ovarian tissue. We found a variation in the health and in the numbers of the transitional, primary and secondary follicles. Of note, we found that both cultured and uncultured samples selected by neutral red (NR) - stain for assessment of follicles

survival showed higher numbers of healthy follicles. Our study was the first study of this collaboration towards optimisation of culture systems for in vitro culture and maturation of ovarian tissue. The present study reported a new classification system of follicles health in human cryopreserved ovarian tissue, based on the presence/absence of pyknosis in granulosa cells, pyknosis in oocytes and the presence or absence of shrunken cytoplasm. Further details and discussion appears in appendix 22 **(Walker et al, 2019, Appendix 22)**.

We recently submitted another manuscript (**Bjarkadottira, et al. 2020, Appendix 23**) analysing different culturing conditions of human ovarian cortical tissue to maximise the follicle survival. We investigated the effect of different culture media and media volumes and dish permeability on health and development of cultured follicles. We found that culturing follicles in low volume conditions had significantly higher odds of being graded as healthy follicles. The alpha MEM (Minimal Essential Medium) medium in a low volume resulted in the highest proportion of healthy follicles and higher rates of development into multi-layered follicles, **see Figure 5 Appendix 23**.
In view of future studies within this collaboration scheme, there are two directions that I intend to further explore- the first is the direction of reconstruction of a bioengineered ovary, based on three dimensional culturing of ovarian tissue to support the in vitro culture of ovarian tissue and folliculogenesis, towards the development of in vitro matured oocytes. There are indications in the literature that three dimensional culture systems that resemble the in vivo three-dimensional structure and niche may promote and support follicular growth and development **(Xu, et al., 2009, Higuchi, et al., 2015)**. In addition by adding three-dimensional motifs of the extracellular matrix, ie RGD (Arginylglycylaspartic acid) components we may mimic the in vivo niche and thus support the development of the follicles. I have applied and got the ethical approval for such a study as shown in A**ppendix 20.** In addition to further studying the in vitro culturing of follicles, this project aims also to be the basis of a bioengineered ovary intended for autotransplantation, where the ovarian tissue will be supported by biofabricated elements including synthetic components to give the tissue a three dimensional ecosystem with supportive components and possibly neo angiogenesis factors or bio fabricated blood vessels.

Another direction of the current research scheme, is investigating the feasibility of reaggregated ovaries of cryopreserved human ovarian cortical tissue, as a mechanism of isolating the somatic supportive cells and the germ cells, followed by reaggregating both components that create follicles-like shapes that

could reconstitute the ovarian ecosystem and function. Reaggregated ovaries (ROs) were initially reported by the Eppig group (**Eppig and Wigglesworth, 2000**). Eppig described a method for the reaggregation of chimeric ovaries by exchanging the germ cells and somatic cells compartments of rat and mouse ovaries. The ROs were rafted beneath the renal capsules in ovariectomized SCID mice. They then demonstrated development of follicles with the normal morphological characteristics. Species specific rat and mouse oocytes developed within follicles composed of somatic cells exclusively of the other species. Rat oocytes showed progress into metaphase II when they were removed from follicles and cultured in vitro. Mice oocytes progressed to mature eggs and underwent fertilization and in vitro development and carried on to term pregnancies. The plan is to adapt this ROs technology to develop mature human oocytes in vitro. ROs may possibly develop into functional artificial ovaries leading to de novo development of follicles that can be either cultured in vitro to create mature eggs or autografted for in vivo development of mature eggs. This is an interesting future direction that I would like to develop and this was added to the in vitro culture ethical approval, further details appear in (**Appendix 21).**

6. Future Horizons

Setting up the ovarian tissue cryopreservation programme in Oxford was met with regulatory, ethical, clinical and biomedical challenges. We were lucky to get the first UK licence as approved by both HTA and HFEA, to set up the ovarian tissue cryopreservation programme and offer the service for all patients around the country, together with the more common and classical options of embryo and egg freeze. Other groups in the UK were doing this as part of research-based study and later on got the licences for clinical application of the technology, i.e. Edinburgh group. Later on, we sought to introduce testicular tissue bio-banking as the infrastructure and the licence could be further extended.

A draft of a manuscript that intends to discuss these aspects is enclosed in **Appendix 18**. It also summarizes the first 100 referrals to the programme. The programme has progressed from earlier stage to offer the service not only for the local and regional population, but also to accept referral and even offer a spoke and hub model for patients.

The establishment of the programme has been instrumental in setting up research programmes to further study the different challenges and unanswered questions in the field and consequently to further develop the technique. The first step was to commence on research projects in the field of in vitro culture of follicles and ovarian tissue. I built up a collaboration with the bioengineering laboratory at Oxford University to advance the work on 3-D culture systems of follicles and ovarian tissue. In the coming years I would like to focus on this area and try to work on bioengineering technologies to advance the neoangiogenesis that is important for the autografts reimplantation. Another direction- to try and advance the in vitro culture technique in the direction of Reaggregated Ovaries utilisation. In this technique, the ovarian tissue is to be cellularized and then separation of somatic and germ cells and then reconstitution of the follicular structures towards in vitro maturation or reimplantation of the reaggregated follicles in vivo.

In recent years, a new innovative technique was described by Kawamura for patients suffering from premature ovarian insufficiency (POI)(**Zhai et, 2016).** This technique is called - in Vitro activation and it includes the harvesting of ovarian slices and then exposure for PTEN (Phosphatase and Tensin homolog) inhibitors before reimplantation in POI patients. Kawamura has described a series of pregnancies and live births demonstrating thus its safety in these patients. As preliminary results have shown positive outcomes that could possibly be replicated and offered to patients within the UK, I am

working on its introduction in the UK, to firstly offer it to the relevant patients and secondly to use it as a basis for more basic biomedical research to develop it and study its safety.

7. **Conclusions:**

The importance of this work which extends over a long period is the need to address the ever growing need to provide cancer patients with fertility cryopreservation options. As has been demonstrated, during our work which spread over a decade, we were lucky to achieve the first UK licence to set up the first ovarian tissue cryopreservation programme and offer the service for all patients around the country, together with the more common and classical options of embryo and egg freeze. The IVM programme offers an independent standalone or an adjunct fertility preservation modality in addition to other modalities, which could offer unique advantages to a certain group of patients. I presented the different contributions that I published during the years that consolidated our knowledge of the different modalities. Our work on the basic science of low responders which showed that there was no an inherent deficiency in StAR enzyme - a rate limiting factor in steroidogenesis, in addition to the fact that the normal mitochondrial function as demonstrated by the JC-1 stain was demonstrated and quoted by the pertinent literature. This work was the basis for interventions to transplant autologous mitochondria from granulosa cells into oocytes from low responders. We solidified the safety of using aromatase inhibitors in IVF patients and it is currently commonly used in different IVF units all over the world. We contributed to the literature in the area of morphokinetic markers of the embryos which is instrumental in accurately informing the patients of the implantation and clinical pregnancy potentials in the future. This is important in order to ensure that significant fertility preservation potential has been achieved by way of embryo freeze. We showed the relative safety of performing egg collections in patients with haemostatic abnormalities predisposing them to bleeding complications. Occasionally, oncologic patients present with bleeding tendency as a result of thrombocytopenia and reassuring patients that oocyte reuptake is a safe option is of paramount importance. Our study in the random start ovulation induction in cancer patients, has been quoted and embodied in the ESHRE guidelines of ovulation induction. Its importance stems from the finding that it offers an equi effective ovarian stimulation with a similar egg numbers harvested without the need to waste precious time waiting for a specific time in the menstrual cycle.

Our different works in IVM have richly contributed to the adoption of IVM as an alternative method to achieve mature eggs and it was instrumental in offering this modality to patients undergoing ovarian

tissue cryopreservation. This utilisation of IVM will be the subject of a separate and independent publication in the near future, as there are only a small number of studies in this area.

The setting up of the ovarian tissue bank was the culmination of a longstanding effort that I led from 2008 till 2013 and nowadays it provides ovarian tissue preservation to both regional and supraregional patients. The infrastructure of the tissue processing and freezing has been utilised to start freezing testicular tissue in prepubertal oncologic boys.

The ovarian tissue biobanking enabled us to expand the scope of research beyond the clinical aspects into basic science projects. My main focus is in the field of in vitro culturing follicles and ovarian tissue bioengineering. We started with our first publication in this area demonstrating the variation of the follicular stages in the frozen thawed ovarian tissue slices.

In the near future, I intend to develop this research in the direction of optimising the in vitro culture of ovarian tissue or follicles, the utilisation of three dimensional culturing and the RO option. As for the future, I would like to work to introduce the IVA technology for patients with POI which will enable me to study the pathophysiology of folliculogenesis in these patients. I am currently in contact with the HTA and the HFEA in an attempt to achieve the licensing needed to commence on this innovative technology.

References:

References **in bold** are my contributions to the body of the published work submitted in evidence for the award of PhD by Publication.

Anderson RA, Mitchell RT, Kelsey TW, Spears N, Telfer EE, Wallace WH. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol. 2015. 3:556-67.

Ataman LM, Ma Y, Duncan FE, Uzzi B, Woodruff TK. Quantifying the growth of oncofertility. Biol. Reprod. 2018. 99:263-265.

Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J. Clin. Oncol. 2008. 26:2630-5.

Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijeyaratne CN, Stener-Victorin E, Fauser BC, Norman RJ, Teede H. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. Hum. Reprod. Update. 2016. 22:687-708.

Ben-Haroush A, Wertheimer A, Klochendler E, Sapir O, Shufaro Y, Oron G. Effect of letrozole added to gonadotropins in controlled ovarian stimulation protocols on the yield and maturity of retrieved oocytes. Gynecol Endocrinol. 2019. 35(4):324-327.

Bergeron ME, Child T, Fatum M. In vitro maturation and surrogacy in patients with vascular-type Ehlers-Danlos syndrome--a safe assisted reproductive technology approach. Hum. Fertil. (Camb.). 2014. 17:141-4. Appendix 12.

Biljan MM, Hemmings R, Brassard N. The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. Fertility and sterility. 2005. 84:S95.

Bjarkadottira BD, Walkera CA, Fatum M, Lane S and Williams SA. Analysing methods of cryopreserved human ovarian cortical strip culture to maximise follicle survival. Submitted to J. Reprod. Fertil. Appendix 23.

Blumenfeld Z. Fertility Preservation in Women With Malignancy: Future Endeavors. Clin. Med. Insights Reprod. Health. 2019. 13:1179558119872490. doi: 10.1177/1179558119872490. eCollection 2019.

Bu Z, Wang K, Dai W, Sun Y. Endometrial thickness significantly affects clinical pregnancy and live birth rates in frozen-thawed embryo transfer cycles. Gynecol. Endocrinol. 2016. 32:524-8.

Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. Curr. Opin. Obstet. Gynecol. 2015. 27:215-21.

Chian RC, Buckett WM, Too LL, Tan SL. Pregnancies resulting from in vitro matured oocytes retrieved from patients with polycystic ovary syndrome after priming with human chorionic gonadotropin. Fertil. Steril. 1999. 72:639-42.

Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, and Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet. Gynecol. 2002. 100: 665–670.

Coskun S, Jaroudi KA, Hollanders JM, Atared AM, Roca GL.
Recovery and maturation of immature oocytes in patients at risk for ovarian hyperstimulation syndrome. J. Assist. Reprod. Genet. 1998. 15(6):372-7.

Demeestere I, Simon P, Emiliani S, Delbaere A, Englert Y. Orthotopic and heterotopic ovarian tissue transplantation Hum. Reprod. Update. 2009. 15:649-665.

Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue banking activities. J. Assist. Reprod. Genet. 2013. 30: 305-14.

Donnez J, Squifflet J, Van Eyck AS, Demylle D, Jadoul P, Van Langendonckt A, Dolmans MM. Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience. Reprod. Biomed. Online. 2008. 16:694-704.

Donnez J, Jadoul P, Squifflet J, Van Langendonckt A, Donnez O, Van Eyck AS, Marinescu C, Dolmans MM. Ovarian tissue cryopreservation and transplantation in cancer patients. Best pract. Res. Clin. Obstet. Gynaecol. 2010. 24:87-100.

Eppig JJ, Wigglesworth K. Development of mouse and rat oocytes in chimeric reaggregated ovaries after interspecific exchange of somatic and germ cell components. Biol. Reprod. 2000. 63:1014-23.

Fatum M , Becker C, McVeigh E, Davis J, Lukhor K, Turner K, Kennedy S, Child T, Lane S. Introducing a centralised tertiary programme for ovarian tissue cryopreservation in female cancer patients. Biomedical, ethical, regulatory and funding challenges. To be submitted. Appendix 18.

Fatum M, McVeigh E, Child T, Lakhor K, Becker C. 3-D Laparoscopically-Assisted Percutaneous Oocyte Retrieval and Ovarian Tissue Harvesting for Fertility Cryopreservation. J Minim. Invasive Gynecol. 2014. 21: S225. Appendix 15.

Fatum M, McVeigh E, Child T. Combined laparoscopic ovarian tissue cryopreservation and immature oocyte retrieval followed by in vitro maturation and vitrification for fertility preservation: UK pilot study results. ESHRE 2015, Abstract. Appendix 16.

Fatum M and Child T. (2018). Female Endocrinology: Anatomy and Physiology. In Turner HE, Eastell R, Grossman A. (Eds), Oxford *Desk Reference Endocrinology*. (205-6). Oxford. UK: Oxford University Press. Appendix 1.

Fatum M and Child T. (2018) Female Endocrinology: Pathophysiology and Investigation. In Turner HE, Eastell R, Grossman A. (Eds), Oxford *Desk Reference Endocrinology*. (207-8). Oxford. UK: Oxford University Press. Appendix 2.

Fatum M, Yivgi-Ohana N, Melamed-Book N, Shweiky D, Orly J, Simon A, Laufer N. StAR levels and mitochondrial membrane potential in granulosa cells of elderly poor responders. Fertil. Steril. 2009. 91:220-5. Appendix 3.

Fatum M, Bergeron ME, ,Ross C, Ding A, Bhevan A, Turner K, Child T. Rescue In Vitro Maturation in Polycystic Ovarian Syndrome Patients Undergoing In Vitro Fertilization Treatment Who Overrespond or Underrespond to Ovarian Stimulation: Is It A Viable Option? A Case Series Study. Int. J. Fertil. Steril. 2020. 14:137-142. Appendix 13.

Fatum M, ME Bergeron, Ross C, Ding A, Bhevan A, Turner K, Child T. Rescue in-vitro maturation in polycystic ovarian syndrome patients over responding/under responding to ovarian stimulation in in-vitro fertilization treatment: is it a viable option? Fertil. Steril. 2013. 100, S271 - S271. Appendix 14.

Fatum M, Gyo Y, Prus D, Laufer N, Simon A. Is estradiol mandatory for an adequate follicular and embryo development? A mouse model using aromatase inhibitor (anastrozole). J Assist Reprod and Genetics. 2006. 23:407-12. Appendix 4

Fatum M, McVeigh E, Child T. The case for aromatase inhibitors use in oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in breast cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate. Hum. Fertil. (Camb). 2013. 16(4):235-40. Appendix 5.

Fatum M, Ozcan C, Simon A, Lewin A, Laufer N. The safety of ultrasound-guided oocytes pick-up in IVF patients with haemostatic disorders. A case series study. Eur. J. Obstet. Gynecol. Reprod. Biol. 2008. 137:259-61. Appendix 9.

Fatum M and McVeigh E. (2015) Ovarian Tissue Cryopreservation. In Metwally M. and Li TC. (Eds), *Reproductive Surgery in Assisted Conception*. London. UK: Springer. Appendix 6.

Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Mortimer D (eds). Towards Reproductive Certainty: Fertility and Genetics Beyond 1999. UK: Parthenon Publishing Carnforth, 1999:378– 388.

Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2017 Jul 5;7:CD003414. doi: 10.1002/14651858.CD003414.pub3.

Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2010 Jan 20;(1):CD006359. doi: 10.1002/14651858.CD006359.pub2.

Gonda KJ, Domar AD, Gleicher N, Marrs RP. Insights from clinical experience in treating IVF poor responders. Reprod. Biomed. Online. 2018. 36(1):12-19.

Green DM, Kawashima T, Stovall M, Leisenring W, Sklar CA, Mertens AC, Donaldson SS, Byrne J, Robison LL. Fertility of female survivors of childhood cancer: a report from the childhood cancer survivor study. J. Clin. Oncol. 2009. 27:2677-2685.

Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, Child T. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case–control study of 194 treatment cycles. Fertil. Steril. 2012. 98:355-60. Appendix 11.

Grynberg M, Poulain M, le Parco S, Sifer C, Fanchin R, Frydman N. Similar in vitro maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum. Reprod. 2016. 31:623-9.

Higuchi CM, Maeda Y, Horiuchi T, Yamazaki Y. A Simplified Method for Three-Dimensional (3-D) Ovarian Tissue Culture Yielding Oocytes Competent to Produce Full-Term Offspring in Mice. PLoS One. 2015. Nov 16;10(11):e0143114. doi: 10.1371/journal.pone.0143114. eCollection 2015.

Hsueh AJ, Kawamura K, Cheng Y, Fauser BC. Intraovarian control of early folliculogenesis. Endocr. Rev. 2015. 36(1):1-24.

Jadoul P, Dolmans MM, Donnez J. Fertility preservation in girls during childhood: is it feasible, efficient and safe and to whom should it be proposed? Hum. Reprod. Update. 2010. 16: 617-630.

Jaroudi KA, Hollanders JM, Elnour AM, Roca GL, Atared AM, Coskun S.
Embryo development and pregnancies from in-vitro matured and fertilized human oocytes. Hum. Reprod. 1999. 14:1749-51.

Jeve YB, Gelbaya T, Fatum M. Time to consider ovarian tissue cryopreservation for girls with Turner's syndrome: an opinion paper. Hum Reprod Open. 2019 Jun 20;2019(3):hoz016. doi: 10.1093/hropen/hoz016. eCollection 2019. Appendix 19.

Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. N. Engl. J. Med. 2009. 360:902-11.

Kedem A, Yerushalmi GM, Brengauz M, Raanani H, Orvieto R, Hourvitz A, Meirow D. Outcome of immature oocytes collection of 119 cancer patients during ovarian tissue harvesting for fertility preservation. J. Assist. Reprod. Genet. 2018. 35:851-856.

Knight S, Lorenzo A, Maloney AM, Srikanthan A, Donen R, Greenblatt E, Gupta A. An approach to fertility preservation in prepubertal and postpubertal females: a critical review of current literature. Pediatr. Blood Cancer. 2015. 62:935-9.

Ladanyi C, Mor A, Christianson MS, Dhillon N, Segars JH. Recent advances in the field of ovarian tissue cryopreservation and opportunities for research. J. Assist. Reprod. Genet. 2017. 34:709-722.

Lambalk CB, Banga FR, Huirne JA, Toftager M, Pinborg A, Homburg R, van der Veen F, van Wely M. GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type. Hum. Reprod. Update. 2017. 23:560-579.

Lawrenz B, Banys M, Henes M, Neunhoeffer E, Grischke EM, Fehm T. Pregnancy after breast cancer: case report and review of the literature. Arch. Gynecol. Obstet. 2011. 283:837-43.

Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, Christman GM, Huang H, Yan Q, Alvero R, Haisenleder DJ, Barnhart KT, Bates GW, Usadi R, Lucidi S, Baker V, Trussell JC, Krawetz SA, Snyder P, Ohl D, Santoro N, Eisenberg E, Zhang H; NICHD Reproductive Medicine Network. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N. Engl. J. Med. 2014. 371:119-29.

Levine JM1, Kelvin JF, Quinn GP, Gracia CR., Infertility in reproductive-age female cancer survivors. Cancer. 2015. 121:1532-9.

Lewin A, Pisov G, Turgeman R, Fatum M, Shufaro Y, Simon A, Laufer N, Revel A, Reubinoff B, Safran A. Simplified artificial endometrial preparation, using oral estradiol and novel vaginal progesterone tablets: a prospective randomized study. Gynecol. Endocrinol. 2002. 16:131-6. Appendix 10a.

Luke B, Brown MB, Missmer SA, Spector LG, Leach RE, Williams M, Koch L, Smith YR, Stern JE, Ball GD, Schymura MJ. Assisted reproductive technology use and outcomes among women with a history of cancer. Hum. Reprod. 2016. 31:183–9.

Mahajan N, Sharma S.The endometrium in assisted reproductive technology: How thin is thin? J. Hum. Reprod. Sci. 2016. 9:3-8.

Manuel SL, Moravek MB, Confino R, Smith KN, Lawson AK, Klock SC, Pavone ME.
Ovarian stimulation is a safe and effective fertility preservation option in the adolescent and young adult population. J. Assist. Reprod. Genet. 2019 Dec 11. doi: 10.1007/s10815-019-01639-y. *[Epub ahead of print].*

Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E, Dor J. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N. Engl. J. Med. 2005. 353:318-21.

Michaeli J, Weintraub M, Gross E, Ginosar Y, Ravitsky V, Eizenman E, Mitrani E, Lebovich M, Laufer N, Kennedy S, Revel A. Fertility preservation in girls. Obstet. Gynecol. Int. 2012;2012:139193. doi: 10.1155/2012/139193. Epub 2012 Feb 16.

Mizrachi Y, Horowitz E, Farhi J, Raziel A, Weissman A. Ovarian stimulation for freeze-all IVF cycles: a systematic review. Hum. Reprod. Update. 2020. 26:118-135.

Moawad NS, Santamaria E, Rhoton-Vlasak A, Lightsey JL. Laparoscopic Ovarian Transposition Before Pelvic Cancer Treatment: Ovarian Function and Fertility Preservation. J. Minim. Invasive Gynecol. 2017. 24:28-35.

Moini A, Lavasani Z, Kashani L, Mojtahedi MF, Yamini N. Letrozole as co-treatment agent in ovarian stimulation antagonist protocol in poor responders: A double-blind randomized clinical trial. Int. J. Reprod. Biomed. 2019. 17:653-660.

Muteshi C, Child T, Ohuma E, Fatum M. Ovarian response and follow-up outcomes in women diagnosed with cancer having fertility preservation: Comparison of random start and early follicular phase stimulation - cohort study. Eur. J. Obstet. Gynecol. Reprod. Biol. 2018. 230:10-14. Appendix 7.

Nayak SR, Wakim AN. Random-start gonadotropin-releasing hormone (GnRH) antagonist-treated cycles with GnRH agonist trigger for fertility preservation. Fertil. Steril. 2011. 96:51-4.

Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, Opsahl M, Rosenwaks Z. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004. 363:837-840.

Oktay K, Harvey BE, Loren AW. Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update Summary. J. Oncol. Pract. 2018. 14:381-385.

Ortega I, García-Velasco JA, Pellicer A. Ovarian manipulation in ART: going beyond physiological standards to provide best clinical outcomes. J. Assist. Reprod. Genet. 2018. 35:1751-1762.

Pereira N, Hancock K, Cordeiro CN, Lekovich JP, Schattman GL, Rosenwaks Z. Comparison of ovarian stimulation response in patients with breast cancer undergoing ovarian stimulation with letrozole and gonadotropins to patients undergoing ovarian stimulation with gonadotropins alone for elective cryopreservation of oocytes†. Gynecol Endocrinol. 2016. 32:823-826.

Revel A, Revel-Vilk S. Fertility preservation in young cancer patients. J. Hum. Reprod. Sci. 2010. 3:2-7.

Revel A, Laufer N, Ben Meir A, Lebovich M, Mitrani E. Micro-organ ovarian transplantation enables pregnancy: a case report. Hum. Reprod. 2011. 26: 1097-1103.

Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. Reprod. Biomed. Online. 2011. 22:162-171.

Salama M, Anazodo A, Woodruff T. Preserving fertility in female patients with hematological malignancies: a multidisciplinary oncofertility approach. Ann Oncol. 2019. 30:1760-1775.

Schenker JG and Fatum M. Should Ovarian Tissue Cryopreservation be Recommended For Cancer Patients ? J. Assist. Reprod. and Genetics. 2004. 21:377-378. Appendix 17.

Schmidt KL, Andersen CY, Loft A, Byskov AG, Ernst E, Andersen AN. Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. Hum. Reprod. 2005. 20:3539-46.

Shufaro Y, Lebovich M, Aizenman E, Miller C, Simon A, Laufer N, Saada A. Human granulosa luteal cell oxidative phosphorylation function is not affected by age or ovarian response. Fertil. Steril. 2012. 98:166-72.

Shufaro Y, Simon A, Laufer N, Fatum M. Thin unresponsive endometrium- a possible complication of surgical curettage compromising ART outcome. J Assist. Reprod. Genet. 2008. 25:421-5. Appendix 10b.

Simon A, Hurwitz A, Zentner BS, Bdolah Y, Laufer N. Transfer of frozen-thawed embryos in artificially prepared cycles with and without prior gonadotrophin-releasing hormone agonist suppression: a prospective randomized study. Hum. Reprod. 1998. 13:2712-7.

Son WY, Henderson S, Cohen Y, Dahan M, Buckett W. Immature Oocyte for Fertility Preservation. Front Endocrinol (Lausanne). 2019 Jul 17;10:464. doi: 10.3389/fendo.2019.00464.

Sonmezer M and Oktay K. Fertility preservation in female patients. Hum. Reprod. Update. 2004. 10:251-266.

Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, Klinger FG. Ovarian damage from chemotherapy and current approaches to its protection. Hum. Reprod. Update. 2019. 25:673-693.

Subira J, Craig J, Turner K, Bevani A, Ohuma E, Child T, Fatum M. Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers. Hum. Fertil. (Camb.). 2016. 19:254-261. Appendix 8.

Subira J, Craig J, Turner K, Bevani A, Ohuma E, Child T, **Fatum M**. Blastocyst expansion and inner cell mass grades are associated with clinical pregnancy in fresh single transfers. Fertil. Steril. 2014. 102, E63.

The ESHRE Guideline Group on Ovarian Stimulation, Ernesto Bosch, Simone Broer, Georg Griesinger, Michael Grynberg, Peter Humaidan, Estratios Kolibianakis, Michal Kunicki, Antonio La Marca, George Lainas, Nathalie Le Clef, Nathalie Massin, Sebastiaan Mastenbroek, Nikolaos Polyzos, Sesh Kamal Sunkara, Tanya Timeva, Mira Töyli, Janos Urbancsek, Nathalie Vermeulen, Frank Broekmans, ESHRE guideline: ovarian stimulation for IVF/ICSI, *Human Reproduction Open*, Volume 2020, Issue 2, 2020, hoaa009, https://doi.org/10.1093/hropen/hoaa009

Thompson SM, Onwubalili N, Brown K, Jindal SK, McGovern PG. Blastocyst expansion score and trophectoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer (eSET): a national study. J. Assist. Reprod. Genet. 2013. 30:1577-81.

Tulandi T, Huang JY, Tan SL. Preservation of female fertility: an essential progress. Obstet. Gynecol. 2008. 112:1160–72.

Turan V and Oktay K. Sexual and fertility adverse effects associated with chemotherapy treatment in women. Expert Opin. Drug Saf. 2014. 13:775–83.

Vassilakopoulou M, Boostandoost E, Papaxoinis G, de La Motte Rouge T, Khayat D, Psyrri A. Anticancer treatment and fertility: Effect of therapeutic modalities on reproductive system and functions. Crit. Rev. Oncol. Hematol. 2016. 97:328-34.

Walker CA, Bjarkadottir BD, Fatum M, Lane S, Williams SA. Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation. Hum. Fertil. (Camb.). 2019. 23:1-11. Appendix 22.

Wallace WH, Smith AG, Kelsey TW, Edgar AE, Anderson RA. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol. 2014. 15:1129-36.

Xu M, West-Farrell ER, Stouffer RL, Shea LD, Woodruff TK, Zelinski MB. Encapsulated three-dimensional culture supports development of nonhuman primate secondary follicles. Biol. Reprod. 2009. 81:587-94.

Yang ZY, Chian RC. Development of in vitro maturation techniques for clinical applications. Fertil. Steril. 2017. 108:577-584.

Zhai J, Yao G, Dong F, Bu Z, Cheng Y, Sato Y, Hu L, Zhang Y, Wang J, Dai S, Li J, Sun J, Hsueh AJ, Kawamura K, Sun Y. In Vitro Activation of Follicles and Fresh Tissue Auto-transplantation in Primary Ovarian Insufficiency Patients. J Clin Endocrinol Metab. 2016. 101(11):4405-4412.

Appendix 1

Fatum M and Child T. (2018). Female Endocrinology: Anatomy and Physiology. In Turner HE, Eastell R, Grossman A. (Eds), Oxford Desk Reference Endocrinology. (205-6). Oxford. UK: Oxford University Press.

Contribution by Fatum M:

Concept

Literature Review

Manuscript Writing and editing

Citation Metrics: N/A as a book chapter

Appendix 2

Fatum M and Child T. Female Endocrinology: Pathophysiology and Investigation. In Turner HE, Eastell R, Grossman A. (Eds), Oxford *Desk Reference Endocrinology*. (207-8). Oxford. UK: Oxford University Press.

Contribution by Fatum M:

Concept
Literature Review
Manuscript Writing and editing

Citation Metrics: N/A as a book chapter

Appendix 3

Fatum M, Yivgi-Ohana N, Melamed-Book N, Shweiky D, Orly J, Simon A, Laufer N. StAR levels and mitochondrial membrane potential in granulosa cells of elderly poor responders. Fertil. Steril. 2009. 91:220-5.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 4

Impact Factor: 3.06

Appendix 4

Fatum M, Gyo Y, Prus D, Laufer N, Simon A. Is estradiol mandatory for an adequate follicular and embryo development? A mouse model using aromatase inhibitor (anastrozole). J Assist Reprod and Genetics. 2006. 23:407-12.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 27

Impact Factor: 2.8

Appendix 5

Fatum M, McVeigh E, Child T. The case for aromatase inhibitors use in oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in breast cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate. Hum. Fertil. (Camb). 2013. 16(4):235-40.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 11

Impact Factor: 1.8

Appendix 6

Fatum M and McVeigh E. (2015) Ovarian Tissue Cryopreservation. In Metwally M. and Li TC. (Eds), *Reproductive Surgery in Assisted Conception*. London. UK: Springer.

Contribution by Fatum M

Concept
Data collection
Manuscript Writing and editing

Citation Metrics: N/A Book Chapter
766 downloads of the online e-book

Appendix 7

Muteshi C, Child T, Ohuma E, Fatum M. Ovarian response and follow-up outcomes in women diagnosed with cancer having fertility preservation: Comparison of random start and early follicular phase stimulation - cohort study. Eur. J. Obstet. Gynecol. Reprod. Biol. 2018. 230:10-14.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 5
Impact Factor: 2

Appendix 8

Subira J, Craig J, Turner K, Bevani A, Ohuma E, Child T, Fatum M. Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers. Human Fertility. 2016. 19:254-261.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 16

Impact Factor: 1.8

Appendix 9

Fatum M, Ozcan C, Simon A, Lewin A, Laufer N. The safety of ultrasound-guided oocytes pick-up in IVF patients with haemostatic disorders. A case series study. Eur. J. Obstet. Gynecol. Reprod. Biol. 2008. 137:259-61.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 7
Impact Factor: 2

Appendix 10a.

Lewin A., Pisov G., Turgeman R., Fatum M., Shufaro Y., Simon A, Laufer N, Revel A, Reubinoff B, Safran A. Simplified artificial endometrial preparation, using oral estradiol and novel vaginal progesterone tablets: a prospective randomized study. Gynecol Endocrinol 2002. 16:131-6.

Contribution by Fatum M

Concept
Data collection
Manuscript Writing and editing

Citation Metrics: 27
Impact Factor: 1.45

Appendix 10b.

Shufaro Y, Simon A, Laufer N, Fatum M. Thin unresponsive endometrium--a possible complication of surgical curettage compromising ART outcome. J. Assist. Reprod. Genet. 2008. 25:421-5.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 69

Impact factor: 2.8

Appendix 11

Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, Child T. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case–control study of 194 treatment cycles. Fertil Steril. 2012. 98:355-60.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics:135
Impact Factor:3.06

Appendix 12

Bergeron ME, Child T, Fatum M. In vitro maturation and surrogacy in patients with vascular-type Ehlers-Danlos syndrome--a safe assisted reproductive technology approach. Hum Fertil (Camb). 2014. 17:141-4.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics:4
Impact Factor: 1.8

Appendix 13

Fatum M, Bergeron ME, ,Ross C, Ding A, Bhevan A, Turner K, Child T. Rescue In Vitro Maturation in Polycystic Ovarian Syndrome Patients Undergoing In Vitro Fertilization Treatment Who Overrespond or Underrespond to Ovarian Stimulation: Is It A Viable Option? A Case Series Study. Int. J. Fertil. Steril. 2020. In press.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: In Press
Impact Factor: 0.94

Appendix 14

Fatum M, ME Bergeron, Ross C, Ding A, Bhevan A, Turner K, Child T. Rescue in-vitro maturation in polycystic ovarian syndrome patients over responding/under responding to ovarian stimulation in in-vitro fertilization treatment: is it a viable option? Fertil. Steril. 2013. 100, S271 - S271.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 5
Impact Factor: 3

Appendix 15

Fatum M, McVeigh E, Child T, Lakhor K, Becker C. 3-D Laparoscopically-Assisted Percutaneous Oocyte Retrieval and Ovarian Tissue Harvesting for Fertility Cryopreservation. Journal of Minimally Invasive Gynecology. 2014 21, S225.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 0 (Abstract)
Impact Factor:2.4

Appendix 16

Fatum M, McVeigh E, Turner K, Ross C, Child T. Combined laparoscopic ovarian tissue cryopreservation and immature oocyte retrieval followed by in vitro maturation and vitrification for fertility preservation: UK pilot study results. ESHRE 2015, Abstract.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: N/A

Appendix 17

Schenker JG and Fatum M. Should Ovarian Tissue Cryopreservation be Recommended For Cancer Patients? J. Assist. Reprod. and Genetics. 2004. 21:377-378.

Contribution by Fatum M

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: 7
Impact Factor: 2.8

Appendix 18.

Fatum M , Becker C, McVeigh E, Davis J, Lukhor K, Turner K, Kennedy S, Child T, Lane S. Introducing a centralised tertiary programme for ovarian tissue cryopreservation in female cancer patients. Biomedical, ethical, regulatory and funding challenges. To be submitted.

Contribution by Fatum M:

Concept
Data collection
Data Analysis
Manuscript Writing and editing

Citation Metrics: N/A to be submitted

Appendix 19.

Jeve YB, Gelbaya T, Fatum M. Time to consider ovarian tissue cryopreservation for girls with Turner's syndrome: an opinion paper. Hum Reprod Open. 2019 Jun 20;2019(3):hoz016. doi: 10.1093/hropen/hoz016. eCollection 2019.

Contribution by Fatum M:

Concept
Literature review
Manuscript Writing and editing

Citation Metrics: 0 (recent publication)
Impact Factor: NA (A new journal)

Appendix 20

Ethical Approval

Appendix 21

Amendment of Ethical Approval

Appendix 22

Walker CA, Bjarkadottir BD, Fatum M, Lane S, Williams SA. Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation.Hum. Fertil. 2019. 23:1-11.

Contribution by Fatum M

Concept
Ethical Approval collection
Critical reading
Manuscript revising and editing

Citation Metrics: 0 (recent publication)

Impact Factor: 1.8

Appendix 23

Bjarkadottira BD, Walkera CA, Fatum M, Lane S and Williams SA. Analysing methods of cryopreserved human ovarian cortical strip culture to maximise follicle survival. Submitted to Journal of Reproduction and Fertility (2020).

Contribution by Fatum M

Concept
Ethical Approval
Patient recruitment
Critical reading
Manuscript revising and editing

Citation Metrics- In submission.

Appendix 24

Appendix 24- UPR16 Form- Research Ethics Review Checklist

6. FEMALE HORMONE METABOLISM

6.1 Anatomy and Physiology

Muhammad Fatum
Tim Child

Introduction:

Human ovaries are cyclically active endocrine organs responsible for the periodic process of egg maturation and ovulation and for the production of the main steroid hormones oestradiol and progesterone. Both functions are tightly meshed with feedback mechanisms to the hypothalamus and pituitary glands in the hypothalamo-pituitary-ovarian (HPO) axis. Other endocrine organs such as the thyroid and the adrenal glands are equally important for normal reproductive functions. This well orchestrated process normally results in regular ovulation and leads to cyclic changes in the endometrium and the predictable monthly menses in adult females.

Anatomy:

The adult human ovaries are oval bodies averaging 14 g each and lie over the ovarian fossae at the postero- lateral pelvic wall and are attached to the posterior leaflet of the broad ligaments by the mesovarium. The ovary consists of three major distinct portions: 1. The outer cortex containing two regions: the outermost region being the single layer of cuboidal epithelium referred to as the surface germinal epithelium and the inner region containing the follicles embedded in stromal tissue derived from mesenchymal cells which have the ability to respond to luteinizing hormone (LH) or human chorionic gonadotropin (HCG). 2. The central medulla consisting of stroma which is derived mainly from mesonephric cells. 3. The rete ovarii (the hilum) in the area of attachment of the ovary to the mesovarium. It contains nerves, blood vessels and the hilar cells which can potentially become active in steroidogenesis.

Follicles:

The follicle represents the basic structural and functional complex in the ovary with respect to oocyte maturation and steroidogenesis. The primordial follicles start to form at 18-20 gestational weeks. Each primordial follicle contains an oocyte arrested at the prophase stage of the first meiotic division, enveloped by a single layer of pregranulosa cells surrounded by a basement membrane. They are embedded in the loose connective tissue of the ovarian cortex. Only a minority of primordial follicles are recruited during a woman's reproductive lifetime, most undergo the process of atresia. The recruitment of primordial follicles into growing follicles induces changes in granulosa cell growth and maturation, as well as follicular structural and functional changes. They typically grow and develop through primary, preantral and antral follicle stages. The early stages of follicular growth are FSH independent and are probably controlled by local intraovarian factors such as anti-mullerian hormone (AMH). Full maturity, as expressed by ovulation, typically occurs in the reproductive age only, as the dominant ovulating follicle is selected during the early days of the same cycle. At 16-20 gestational weeks there are 6-7 million germ cells. At birth, the number of oocytes is nearly 1 million. At the onset of puberty the oocyte number is reduced to about 500,000 and throughout the reproductive life only 300-500 oocytes will be selected to ovulate. The vast majority of eggs are lost in a process of atresia or apoptosis, programmed cell death.

Follicular growth and egg maturation:

The early stages of follicular growth occur over a time period of several preceeding cycles, about 85 days prior to achieving preovulatory status. Reaching this stage, the follicles are either recruited by an FSH dependent process at about the transition time between the preceeding cycle's luteal phase and the early days of the current cycle's follicular phase, otherwise, they become arrested and undergo atresia. Typically a cohort of FSH-dependent 2-6mm antral follicles are recruited by the late luteal phase due to the FSH rise of the preceeding cycle. The average time for the development of the selected dominant follicle to the ovulation stage is 10-14 days.

Reproductive Physiology:

Normal reproductive function with cyclic menses requires the pulsatile secretion of GnRH from the hypothalamus. This pulsatile rhythmic release must be within a critical range of frequency and amplitude for normal control to occur. GnRH has a positive effect on the anterior pituitary resulting in increased gonadotrophin synthesis, storage and pulsatile secretion. Lower GnRH pulse frequencies favour FSH secretion and higher frequencies favour LH secretion. The variation in GnRH pulse frequencies is modulated by the ovarian steroid feedback. Oestradiol increases GnRH pulse frequency whereas elevated progesterone levels decrease it. Oestradiol and progesterone levels are important in determining gonadotrophin regulation: **Oestrogen levels:** low oestradiol levels enhance FSH and LH synthesis and storage, have negative effect on FSH secretion with little effect on LH secretion. However, it is the high oestradiol levels that induce the LH surge at

midcycle**. Progesterone levels:** Low levels enhance the LH response to GnRH and are responsible for the FSH surge at midcycle. The increased level of progesterone in the luteal phase inhibit gonadotrophin secretion by inhibiting hypothalamic GnRH pulses and inhibiting the pituitary response to GnRH. It is the raised progesterone levels at the end of the luteal phase that decrease GnRH pulsatile frequency, leading to preferential FSH secretion in the late luteal phase and the start of the next ovarian cycle with follicular cohort recruitment.

GnRH pulsatility is also modulated by neurotransmitters such as dopamine, noradrenaline, endorphins, kisspeptins and others. The half life of GnRH is short (2-4 min) as it is degraded rapidly by peptidases in the hypothalamus and the pituitary gland.

Gonadotrophs are the target cells of GnRH at the level of the anterior pituitary gland and are responsible for the synthesis, storage, activation and secretion of LH and FSH. Each gonadotrophin is a heterodimer and is made of two peptide subunits termed alpha and beta. The alpha subunits are structurally identical but the beta subunits are unique and confer the specific activity. During the late luteal phase FSH levels start to rise and reach a peak around day 3 of menstruation in the next cycle. This causes FSH-dependent antral follicular recruitment and growth, granulosa cell proliferation and differentiation and aromatase action and oestrogen production and inhibin B secretion. The last two hormones exert negative feedback at the hypothalamus and the pituitary. FSH induces LH receptors within the dominant follicle. Oestradiol levels derived from the dominant follicle increase steadily and exert suppressive feedback on FSH release leading to lack of support and consequent atresia of the non-dominant follicles. The mid-follicular rise in estradiol leads to a switch from negative to positive feedback on LH release resulting to the mid-cycle surge. This surge lasts 36-48 hours and is responsible for the resumption of oocyte meiotic maturation, triggering of ovulation, luteinisation of granulosa cells and synthesis of progesterone and prostaglandins within the follicle.

Two-cell, two- gonadotrophin theory for ovarian steroidogenesis:

Oestradiol and progesterone are the main steroid hormones secreted by the ovaries. Ovarian steroidogenesis in the follicles takes place through:

1. LH action on theca cells mediated largely by cyclic AMP, StAR and SF-1 leading to androgen synthesis, predominantly androstenedione, which then diffuses to the granulosa cells.

2. FSH action on granulosa cells through cyclic AMP, LRH-1 and/or SF-1 binding activity, and promotion of aromatase expression in granulosa cells and subsequent oestradiol formation by aromatisation of androstenedione and other androgens.

Oestradiol is the most important oestrogen - its main functions are endometrial development and triggering of the mid-cycle LH surge leading to follicular rupture and ovulation. In addition, the suppression of FSH secretion by negative oestradiol feedback is key to preventing multi-follicular development in the mid-follicular phase once a single dominant follicle has been recruited.

Oestradiol levels rise rapidly after menstruation to reach a peak in the late follicular phase which induces the mid-cycle LH surge. During the luteal phase oestradiol is produced by the corpus luteum; in the absence of an endogenous rise in hCG from an implanting embryo the corpus luteum involutes leading to a sharp decline in oestradiol (and progesterone) levels and consequent menstruation.

The granulosa lutein cells have high levels of LH receptors and produce progesterone, the main luteal phase hormone. Progesterone stimulates secretory changes in the endometrium that are critical for achieving an environment receptive for embryo implantation. Progesterone levels rise following ovulation and decline with the demise of the corpus luteum before menstruation. Peak progesterone levels are reached in the mid-luteal phase and a blood sample (taken 7 days before the estimated day of the following menstrual bleed) is commonly used to confirm ovulation.

Extraovarian steroidogenesis:

Oestradiol production can take place in peripheral tissues such as subcutaneous fat and skin fibroblasts. Peripheral tissue aromatase is responsible for aromatisation of androstenedione resulting in the production of the weaker oestrogen, oestrone. Oestrone is further metabolised and converted to the more biologically active oestradiol in target tissues such as the breast and endometrium. This extra-ovarian production is potentially clinically important in obese women because of the increased mass of fat tissue.

Peptide hormones produced by the ovary:

The ovaries produce a number of peptides that can act in an autocrine, paracrine or endocrine manner. These peptides include numerous cytokines, growth factors and other regulatory proteins such as inhibin, activin and follistatin that are produced by granulosa cells under the control of FSH and LH and take part in the HPO feedback loops. For further reading see the suggested references.

Further reading

Wass JAH, Stewart Paul M. Oxford textbook of endocrinology and diabetes. 2nd edition.Oxford University Press. Oxford. 2011.

Larsen P Reed. Williams Textbook of endocrinology.10th edition. Saunders. Philadelphia, Penn. 2003.

Speroff, L, Glass, RH, Kase, NG, Million, L. Clinical gynecologic endocrinology and infertility. 6th edition. Lippincott Williams & Williams. Baltimore. 1999.

Strauss JF, Barbieri RL. Yen & Jaffe's Reproductive Endocrinology. 6th Edition. Saunders. 2009

6.2a Investigation

Muhammad Fatum

Tim Child

Introduction:

Female reproductive hormone dysfunction is often manifested by disruption of the regular cyclic menses, oligo/anovulation, infertility or a presentation of clinical hyperandrogenism. History taking and physical examination are still the mainstay of any diagnostic workup. Based on clinical presenting symptoms and the physical signs obtained, further investigative workup including biochemical, hormonal and genetic tests and imaging studies can then be used to make a diagnosis.

History:

A detailed history should be taken with the aim of understanding the main presenting complaints including guided questions to assess the possible underlying hormonal disruptions. Thorough knowledge of the physiological effects of the different female hormones is critical for establishing the correct diagnosis. Possible involvement of the hypothalamic-pituitary-ovarian axis (HPO axis) as well as non-HPO axis glands e.g. the adrenal or the thyroid, should be thoroughly evaluated by organ-targeted questions.

The history should include careful evaluation of the menses, their regularity, frequency, duration and quantity of bleeding, age of menarche and the relationship to puberty. Other areas include: past medical and surgical history, intercurrent systemic illnesses or use of medications; primary versus secondary amenorrhea; galactorrhoea and/or visual disturbances; clinical presentation of hyperandrogenism, i.e. hirsutism, acne or signs of virilisation; the gradual versus sudden onset of hirsutism; evaluation of reproductive and obstetric histories; weight and changes, eating disorders and related complaints; physical activity; hot flushes; fertility history and previous contraception. Family history may prove important.

Physical examination:

The physical examination should aim to elicit evidence of abnormality in the primary endocrine organ (eg the thyroid gland) and/or the target organ (eg the skin). Body mass index should be calculated for patients, the severity of hirsutism and acne if indicated assessed, and signs of virilisation i.e. clitoromegaly, voice deepening or hair loss looked-for. Physical examination of the breasts and Tanner staging is undertaken if indicated. Careful gynaecological examination with attention to any vulvovaginal, cervical, uterine and adnexal lesions or pathologies is undertaken.

Based on careful history taking and physical examination, a tentative differential diagnosis is offered. The final diagnosis is most often established or confirmed with completion of the laboratory work-up including biochemical and/or genetic tests. The different tests that are routinely used in the evaluation of female hormonal metabolism dysfunction are discussed below. A structured detailed approach and specific algorithms for the different pertinent diagnoses will be dealt with separately in the relevant chapters.

Biochemical:

An early-follicular phase day 2-5 hormonal profile is the initial step in a female endocrine workup. The test can be taken on a random day in a woman with absent or very irregular menses. If the patient is already on hormonal treatment (ie the COCP) then blood tests should be taken on the 7th off-pill day. These tests will commonly include: FSH, LH, total and free testosterone, prolactin, TSH and free T4. Based on the clinical presentation and the results of the initial workup further tests are arranged as necessary. The differential diagnosis and the stepwise diagnostic workup will be dealt with in more detail in the relevant chapters (chapter 5.1 for PCOS, chapter 3 for prolactinoma/galactorrhoea, chapter 3 for hirsutism/hyperandrogenism, chapter 5.4.6 for ovarian failure).

The FSH and LH levelss are mainly used to differentiate between primary and secondary ovarian dysfunction. Abnormal tests should be repeated after at least one month to confirm the results. Table 6.1 shows a classification of oligo/amenorrhoea, common causes and hormonal profile.
<Table 6.1 here>
Total and free testosterone are the initial tests in hyperandrogenism states. Further tests may be then sent if abnormal results are achieved as follows:

DHEAS and Androstenedione: usually tested if the serum testosterone is greater than 5 nmol/L, in the presence of a rapidly progressive hyperandrogenic state or in cases of virilisation. Androstenedione is elevated in both ovarian and adrenal aetiologies. DHEAS is a useful hyperandrogenic adrenal marker. In cases of adrenal tumours it

is in excess of >20 micromol/L.

17-Hydroxyprogesterone (taken at 8:00 am) is measured to screen for late-onset congenital adrenal hyperplasia (CAH). It is indicated when testosterone levels are greater than 5nmol/L or in cases of virilisation. The blood sample should be taken in the follicular phase of the menstrual cycle to avoid false positive results that may occur in the luteal phase since it is also secreted by the corpus luteum.

17-Hydroxyprogesterone is measured 60 min after intravenous ACTH, and cortisol (8:00 am) measured after 1 mg dexamethasone at midnight if the 17-Hydroxyprogesterone (8:00 am) screening test is abnormal. Typically, an exaggerated rise in 17-hydroxyprogesterone is seen in non-classic CAH. Most patients have levels of >45 nmol/L. Levels <30nmol/L post-ACTH rule out the diagnosis. Levels 30-45 nmol/L suggest heterozygosity or non-classic CAH. Abnormal levels should be confirmed by genotyping.

Depending on the clinical context and degree of suspicion of Cushing's syndrome, blood tests for free cortisol or the over-night dexamethasone suppression testing are used.

Prolactin is measured to exclude hyperprolactinemia as a cause of galactorrhoea, ovulatory dysfunction leading to oligo/amenorrhoea or infertility. Assays for macroprolactin should be used in patients with hyperprolactinaemia and regular ovulatory cycles.

TSH and free T4 are the screening tests for thyroid dysfunction, especially if either hypothyroidism or hyperthyroidism is clinically suspected.

Progesterone challenge test: progesterone withdrawal bleeding is induced using 10mg oral medroxyprogesterone acetate BD for 7 days. If a bleed ensues after progesterone administration is stopped, then there is evidence of adequate oestrogen priming of the endometrium and a normal outflow tract.

Dyslipidaemia and impaired glucose tolerance screening: as PCOS patients are at higher risk of cardiometabolic syndrome with predisposition to hypertriglyceridaemia, hypercholesterolaemia, low HDL cholesterol and impaired glucose tolerance, screening blood tests for blood lipids and fasting glucose are indicated in PCOS patients, especially if they have other metabolic risk factors. If the fasting glucose is abnormal, the glucose challenge test should follow.

Genetic investigation:

Karyotype:

The main indication for chromosomal analysis is premature ovarian insufficiency (POI). Women presenting with hypergonadotrophic hypogonadism below the age of 40 should be karyotyped. Turner syndrome and other X chromosomal abnormalities are responsible for the majority of POI.

Fragile X syndrome:

Testing for FRAXA is indicated in POI in the UK. FMR1 should ideally be tested for in all cases of premature ovarian insufficiency. Several other genes are potentially implicated in POI with variable degrees of evidence.

Levels of steroidogenic acute regulatory protein and mitochondrial membrane potential in granulosa cells of older poor-responder women

Muhammad Fatum, M.D.,[a] *Yivgi-Ohana Natalie, Ph.D.,*[b] *Shveiky David, M.D.,*[a] *Orly Joseph, Ph.D.,*[b] *Alexander Simon, M.D.,*[a] *and Neri Laufer, M.D.*[a]

[a] IVF Unit, Department of Obstetrics and Gynecology, Hadassah Medical Center; and [b] Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

Objective: To compare mitochondrial function in granulosa cells obtained from older (>40 y) low-responder IVF patients with that of young (<35 y) good-responder patients.
Design: Prospective laboratory research.
Setting: In vitro fertilization unit in a university hospital.
Patient(s): Twenty patients undergoing IVF treatment cycles.
Intervention(s): Ultrasound guided oocytes pick-up.
Main Outcome Measure(s): Mitochondrial function examined by using JC-1 stain for the mitochondrial membrane potential in granulosa cells of both groups and Western blots for assaying and quantification of steroidogenic acute regulatory protein (StAR) and p450scc (side-chain cleavage).
Result(s): The number of granulosa cells per follicle differed between the two groups, with fewer granulosa cells isolated in the older low-responder women, compared with in the young, normal responders who were the control women. Trypan blue–negative cells showed similar undisturbed mitochondrial membrane potential, and similar ratios of apoptotic granulosa cells were observed in the two groups. In addition, there was no difference in StAR and P450scc protein levels between the two groups.
Conclusion(s): Our results demonstrate a significant decrease in the number of total aspirated granulosa cells per follicle in older, poor-responder women, which probably explains the reduced hormonal production by those follicles. However, those cells demonstrate normal mitochondrial membrane potential as well as similar levels of StAR, P450scc, and de novo steroid hormone synthesis in the two groups of patients. Our results do not support mitochondrial dysfunction as a main mechanism of reproductive aging. (Fertil Steril® 2009;91:220–5. ©2009 by American Society for Reproductive Medicine.)

Key Words: Granulosa cells, older poor responder, mitochondria, steroidogenesis

The delay in childbearing in most Western societies is an important societal change, contributing to an increasing incidence of subfertility. Increasing numbers of women decide at a young age not to have children, but they change their minds at a later age. Others deliberately delay childbearing to a period in life when having children appears to be more compatible with their chosen lifestyle (1). The progressive increase in reproductive aging caused by a delay in childbearing contributes significantly to the growing incidence of unwanted subfertility. Therefore, female fertility and its age-related decline is becoming a major problem that still lacks efficient treatment.

In one study, the proportion of infertile women increased progressively, from 6% in the 15- to 24-year age group to >30% in the 35- to 44-year age group (2). Other studies have shown that the probability of achieving a pregnancy within 1 year is significantly decreased in women >35 years of age (3, 4). A British study based on >35,000 IVF cycles demonstrated that female age is by far the most important determinant of success (5) and that live-birth rates per embryo transfer dropped from 24% for women <30 years of age to 8% in women 40–44 years of age and 3.5% in women >45 years of age. The age-related decline of female fertility consists of two components: the first is the decreased monthly probability of conception, and the second is the increased probability that a pregnancy will terminate after conception or implantation (i.e., embryo loss, pregnancy loss, fetal loss, or spontaneous abortion).

The prevailing concept of human reproductive aging assumes that the age-dependent loss of female fertility is dictated by the decline of both the quantity and quality of the oocyte and follicle pool. Numbers of follicles decline exponentially, with a marked increase in the rate of disappearance

Received June 18, 2007; revised and accepted October 3, 2007.
Supported, in part, by a grant from the Hadassah Women's Health Research Fund.
Reprint requests: Fatum Muhammad, M.D., IVF Unit, Department of Obstetrics and Gynecology, Hadassah Ein-Kerem, POB 12124, Jerusalem 91121 Israel (E-mail: muhammadf@ekmd.huji.ac.il).

0015-0282/09/$36.00
doi:10.1016/j.fertnstert.2007.10.027

from age 37–38 years. From the millions present before birth, only 300,000 follicles are left at the beginning of puberty, and subsequently, hundreds vanish every month. Below a critical number of some thousands, reached at an age of 45–46 years, the menstrual bleeding pattern becomes irregular (6), and when the menopause is reached at a mean age of 51 years, the supply is reduced to a $\leq$1000 or follicles, which is a number insufficient to sustain the cyclic hormonal process necessary for menstruation (7). In addition, chromosomal aneuploidy is considered to be the major cause of the age-related decline of oocyte quality. The frequency of chromosome abnormalities at conception increases rapidly with increasing maternal age, so that the majority of embryos are chromosomally abnormal in women approaching 40 years of age. This has been confirmed by investigations into the chromosomal status of IVF embryos that have demonstrated that the majority of embryos derived from women >37 years of age are chromosomally abnormal and contain all possible combinations of both monosomies and trisomies (8, 9). Accumulating studies show complex interactions between oocytes and granulosa cells that are reflected also by changes in the follicular fluid content (10–12). Age-related changes leading to an increased probability of meiotic nondisjunction (e.g., accumulation of oxidative stress) may well be mediated by subtle disturbances of granulosa cell function (1).

The free-radical theory is one of the most comprehensive theories of aging. It suggests that living organisms age because of accumulation of oxygen radical–induced cellular damage and that mitochondrial DNA is a possible target of free-radical attack during the aging process. In recent years, an increasing number of reports have shown that mitochondrial DNA mutations are associated with human aging and mitochondrial diseases (13). Deleterious mitochondrial DNA rearrangements cause cellular energy deficiencies and result in clinical disorders (14). Deficiencies in mitochondrial adenosine triphosphate production may be associated with the impairment of oocyte fertilization. It has been suggested that differences in the amount of adenosine triphosphate generated by mature human oocytes, during IVF, may be related to the fertilization potential and developmental competence of an embryo (15). Another study found that women >38 years of age had granulosa cells that contained a substantial decrease in the level of normal mitochondria, as compared with the case of women <34 years of age (16). Women >38 years of age exhibited an increase in mitochondrial DNA deletions. These deletions may be a result of exposure of mitochondrial DNA to oxygen free radicals generated by several mitochondrial P450 enzymes. In recent years, increased mitochondrial DNA deletions have been demonstrated to facilitate the opening of mitochondrial permeability transition pores via the mitochondrial release of cytochrome *c* and apoptosis-inducing factor, which ultimately leads to the final event of apoptosis (17, 18).

In the present study, we aimed to compare mitochondrial function in granulosa cells of young women (<35 y) who had good response to ovulation induction with that of older women (>40 y) with low response. We examined mitochondrial membrane potential by assay of the steroidogenic enzymes steroidogenic acute regulatory (StAR) protein and cholesterol side-chain cleavage cytochrome P450 (P450scc), by both their levels and expression in granulosa cells of the two different groups.

MATERIALS AND METHODS

The trial was approved by the local institutional research board.

The study included infertile women scheduled for treatment in the IVF unit of the obstetrics and gynecology department at Hadassah Hebrew University Hospital. Four young women (<35) with good response to ovulation induction and 10 older women (>40 y) with low response were recruited. Low responders were defined as women responding with three oocytes at most to ovarian hyperstimulation with exogenous gonadotropins, in the context of IVF treatment. The number of developed follicles and/or number of oocytes retrieved after ovarian stimulation protocol are two of the most important criteria for poor ovarian response. We chose the definition of low response to include those women with fewer than three oocytes retrieved after ovarian stimulation because that is a simple and widely used definition. However, other, different definitions are present in the literature (19).

All women underwent the same pituitary desensitization treatment with decapeptyl (Ferring, Malmo, Sweden), followed by exogenous gonadotropin stimulation with recombinant FSH (Gonal-F; Serono, Herzlia, Israel) in preparation for IVF.

Granulosa cells were isolated from each woman after ovum pickup, separated from red blood cells by using Ficoll separation media, and processed by centrifuge. The follicular fluid from each woman was pooled and preserved at −20°C. Pooled granulosa cells from each patient were counted by a hematocytometer, using trypan blue exclusion dye to determine viability. Granulosa cells were extracted by lysis buffer (RIPA buffer: 150 mM NaCl; 50 mM Tris-HCl, pH 7.5; 1% Triton X-100; 0.5% deoxycholate; 0.1% sodium dodecyl sulfate (SDS); and protease inhibitors cocktail; Sigma; St. Louis, MO). Samples were put on ice for 30 minutes and processed by centrifuge for 2 minutes at 14,000 × *g*. The supernatant was separated, and protein concentration was determined by a modification of the protein assay method of Bradford (20). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer (31 mM Tris-HCl, pH 6.8; 1% SDS; 0.05 mM ethylenediaminetetraacetic acid; 5% glycerol; and 0.003% bromophenol blue) was added to the samples, and after heating for 5 minutes at 95°C, samples were stored at −200°C until use. Samples (20 μg per lane) were electrophoresed on a 10% mini gel by standard SDS–polyacrylamide gel electrophoresis procedures, along with prestained molecular weight markers (MultiMark; Novex, San Diego, CA). Gels were electrophoresed at 30 mA/mm at 40°C by using a running buffer that included 12.5 mM Tris-base, 96 mM glycine, and 0.01% SDS. The proteins

were electrophoretically transferred to Optitran BA-S 85 nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) by using a semidry electrotransfer apparatus (E&K Scientific Products, Saratoga, CA). Protein transfer was conducted for 45 minutes at 3 mA/cm^2 in a modified buffer consisting of 48 mM Tris-base, 39 mM glycine, 0.04% SDS, and 20% methanol. The membrane was blocked by a 30-minute incubation in blocking buffer (PBS buffer containing 0.1% Tween 20 and 5% nonfat dry milk), followed by an overnight incubation with anti-StAR (1:5,000) and anti P450scc (1:5,000). After three washes for 5 minutes each with PBS–Tween buffer (similar to the buffer described in the previous sentence, but without the milk), the membranes were incubated for 2 hours with peroxidase-conjugated goat anti-rabbit IgG (1:10,000 dilution). Specific signals were detected by chemiluminescence by using the LumiGlo substrate (New England BioLabs, Beverly, MA).

Quantitation of chemiluminescence signals on radiograph films was performed as follows: chemiluminescence pseudoautoradiograms were scanned (Astra 4000U; UMAX, Fremont, CA). Quantification of scanned images was performed according to the user manual of the public-domain NIH Image program (National Institutes of Health, Bethesda, MD).

To investigate the status of mitochondrial membrane potentials in the different age groups, granulosa cells were stained using the JC-1 stain and examined by confocal microscopy. For the purpose of JC-1 staining (21), granulosa cells were seeded onto a glass slide (12-mm diameter) that was glued onto the outside of a 35-mm tissue culture dish (Falcon; Becton Dickinson, Plymouth, Devon, United Kingdom) in which a 10-mm hole was drilled at the center. This custom-made dish allows scanning of live cells with an inverted microscope (BioRad 1024 confocal workstation, BioRad, Richmond, CA). To detect dissipated membrane potential in aging mitochondria from low-responder granulosa cells, we used 5 μM JC-1 (Molecular Probes; Eugene, OR), which was added for a 15-minute incubation (37°C) before washing and confocal scanning. The cells were scanned by using a 488-nm wavelength for excitation, and the dual emissions of JC-1 were collected with a 525 ± 20 filter (green) and a 585 long-pass filter (red). Loss of membrane potential results in a concomitant loss of red emission, but the mitochondria remain visible when viewed through the green filter. Therefore, JC-1–stained cells are shown in red and/or red-green overlay. Radioimmunoassay of pooled follicular fluids levels of E_2, T, and P of each woman were performed.

Later, we performed a cytospin of granulosa cells of both groups and stained cells with propidium iodide. We used fluorescent microscopy to study 100 cells of randomly chosen fields from the cytospin slides for apoptotic features.

RESULTS

Patients

Compared with the control group, women in the older low-responder group had significantly higher day 3 FSH levels (in mIU/mL) (9.6 ± 2.4 vs. 5.8 ± 1.7, n = 10, P=.02), lower E_2 levels (in pg/mL) on the day of hCG administration (1,856 ± 1,074 vs. 9,222.5 ± 2,398.2, n = 10, P<.01), a lower number of follicles (3.3 ± 1.2 vs. 12.25 ± 3.3, n = 10, P<.01), a lower number of oocytes (1.5 ± 0.5 vs. 11.75 ± 2.86, n = 10, P<.01), and a lower number of embryos (1.5 ± 0.8 vs. 10.75 ± 2.6, n = 10, P<.01).

Granulosa-Cell Isolation

The number of trypan blue–negative granulosa cells per follicle differed between the two groups with lesser granulosa cells isolated in the study group of older low-responder women, compared with in the control young, normal responders (121,000 ± 30,888 vs. 475,555 ± 73,333 respectively, n = 10, P<.01).

Levels of StAR in Older Poor Responders and Young, Normal-Responder Women

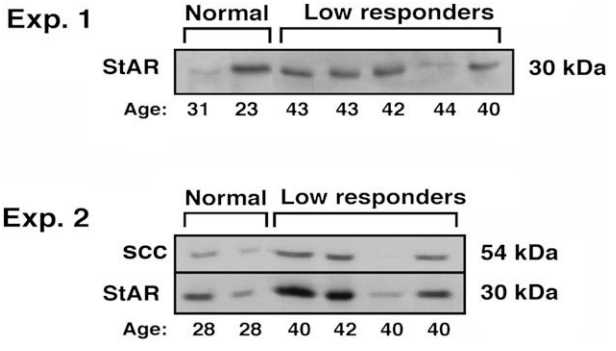
Western blots of nine women in the study group and four of those in the control group are shown in Figure 1. As can be seen, there is no difference in StAR and P450scc protein levels between the two groups. This suggests that StAR and P450scc protein levels do not play a role in granulosa cells' senescence.

Mitochondrial Membrane Potential in Older Poor Responders and Young, Normal-Responder Women

Figure 2A shows the JC-1 staining of the group of young, normal-responder women observed by confocal microscopy. The mitochondria are stained by red and green colors, an evidence of normal mitochondrial membrane potential. Figure 2B shows undisturbed mitochondrial membrane potential in the older low-responder women group.

FIGURE 1

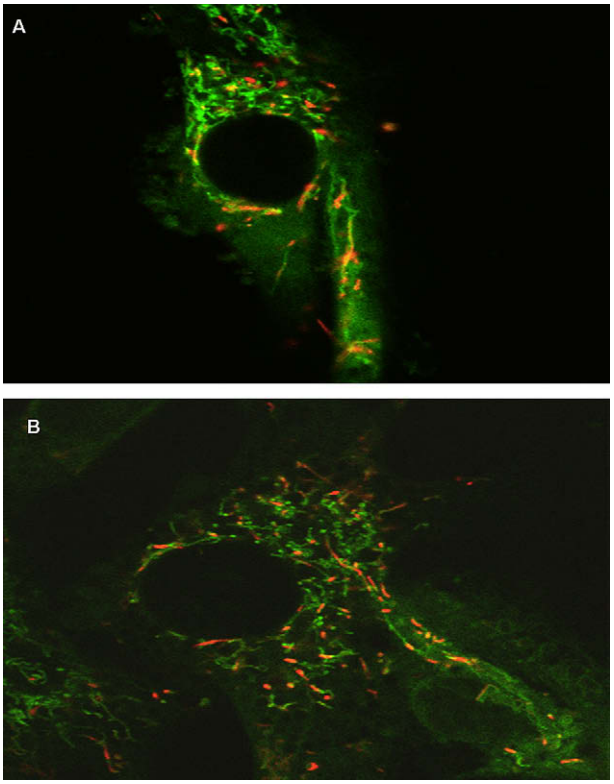
Western blot of StAR and side-chain cleavage enzyme (SCC) levels in older low-responder women compared with young normal-responder women. No difference was seen in StAR and P450SCC levels in the two groups.



Fatum. Granulosa cells in older poor-responder women. Fertil Steril 2009.

FIGURE 2

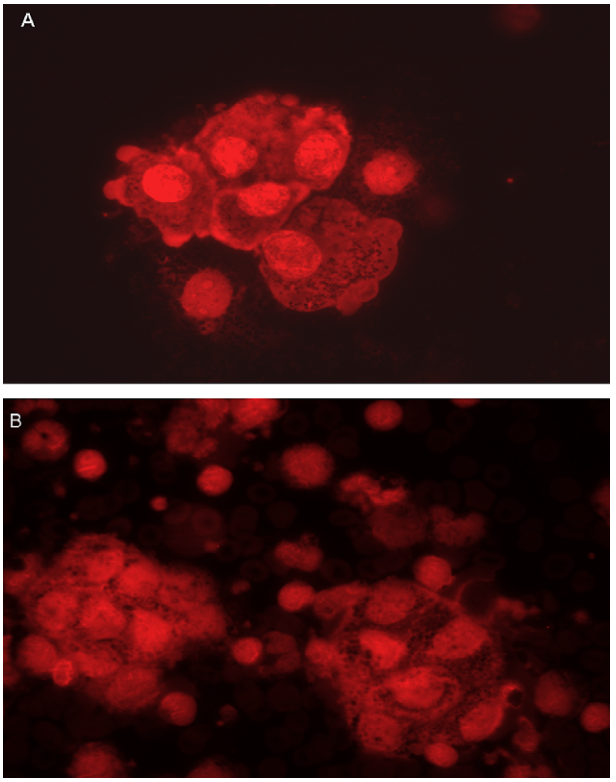
(**A**) Staining with JC-1 of sample from young normal-responder woman as observed by confocal microscopy. (**B**) Staining with JC-1 of sample from poor-responder woman.



Fatum. Granulosa cells in older poor-responder women. Fertil Steril 2009.

FIGURE 3

(**A**) Propidium iodide stain of nuclei of granulosa cells in young good responder. (**B**) Propidium iodide stain of nuclei of granulosa cells in older poor responders.



Fatum. Granulosa cells in older poor-responder women. Fertil Steril 2009.

To further validate the results, we performed a cytospin of granulosa cells of both groups and stained cells with propidium iodide to see whether there was a difference in apoptosis between the two groups. There was no difference in apoptotic-cell percentages in the young good responders, as compared with in the older poor responders (8.6% ± 3.06% vs. 8% ± 1.5%, n = 4, *P*=.9). As shown in Figure 3A and B, we noticed that the majority of granulosa cells are normal and nonapoptotic, with only a minor, and equal, amount being apoptotic in both groups.

Follicular Fluid Steroid Hormone Levels

In an attempt to see whether other downstream steroidogenic enzymes are involved in granulosa cell senescence, we examined the pooled follicular fluids of these women for three target hormones: E_2, T, and P. No significant differences were seen in the levels of the three hormones: E_2 levels ($6.2 \times 10^5 \pm 3.2 \times 10^5$ pM vs. $8.3 \times 10^5 \pm 5.1 \times 10^5$ pM; n = 10, *P*=.3), P levels ($9.4 \times 10^3 \pm 4.1 \times 10^3$ nM vs. $6.9 \times 10^3 \pm 3.6 \times 10^3$ nM; n = 10, *P*=.18), and T levels (25.4 ± 7.8 nM vs. 28 ± 10.2 nM; n = 10, *P*=.56) in the young good responders as compared with in older poor responders. This suggested that the steroidogenic hormonal machinery does not play a central role in the pathophysiology of low response in older women.

DISCUSSION

The present study was aimed at studying age-related mitochondrial function in granulosa cells that were obtained from IVF patients of two different age groups and with different response to stimulation with gonadotropins: older women (≥40 y of age) with low response and young women (<35 y of age) with good response. Two tests for granulosa cell function were examined: the JC-1 stain for the mitochondrial membrane potential and Western blotting technique for StAR and P450scc measurement. Our hypothesis was that during the process of reproductive aging, mitochondrial membrane potential may deteriorate as a result of free-radical accumulation. In addition, we hypothesized that StAR levels would be reduced because the follicular pool is depleted and its endocrinologic steroidogenic machinery is diminished. Steroidogenic acute regulatory protein was our candidate enzyme because its role is to mediate the delivery of cholesterol substrate to the inner mitochondrial membrane, where the

P450scc enzyme complex converts it into pregnenolone, the first steroid synthesized (22). Contrary to these assumptions, we did not find any difference in StAR levels or P450scc levels between the two groups. This observation contradicts the observation described by other investigators (23). In their work using similar luteinizing-granulosa cells isolated after follicular aspiration, Phy et al. (13) found a 17% reduction in StAR protein in patients with a poor response when compared with those with a normal response. These differences may stem from the differing definitions for normal responders and poor responders. Those investigators defined normal responders as those with a threefold or greater increase in P, whereas patients with an increase of less than threefold were categorized as poor responders. However, our definition of low responders is stricter; consequently, this cannot be the explanation.

Because we did not find any difference concerning StAR and P450scc levels between the two groups, it may be assumed that these key enzymes affecting steroid hormone synthesis cannot be key determinants in reproductive aging of granulosa cells of older poor-responder women.

In an attempt to examine other candidate enzymes, we studied the E_2, T, and P levels in the pooled follicular fluid of all follicles punctured at the same oocyte pickup. However, no difference was shown in follicular levels of these hormones. Hence, other downstream steroidogenic enzymes cannot be responsible for the different ovarian response in the two groups.

Similar to these observations, we did not find any differences in JC-1 staining in the two groups. Both groups showed normal mitochondrial membrane potential and normal distribution of mitochondria in the cytoplasm. This result is in contrast to our assumption that with the accumulation of free radicals, the mitochondrial membrane potential may be disrupted. Our results show that mitochondrial membrane potential, as a possible target for free-radical accumulation, does not appear to play a role in aging granulosa cells. One possible interpretation of our inability to show a change of membrane potential is the fact that only the good-quality granulosa cells survived the overnight incubation in culture and were tested by confocal microscopy. It is not unlikely that other necrotic cells degenerated and were washed out. However, the propidium iodide stain consistently has shown similar apoptotic ratios in the two groups. Our results show that apoptotic changes in granulosa cells isolated from low-responder IVF patients are similar to those in younger, good-responder patients. However, in our experiments, we used granulosa cells isolated from follicles that responded to ovarian hyperstimulation, and thus there may have been selection bias that led to the unresponding follicles being dropped out of the study. Granulosa cells from these unresponding follicles may differ from granulosa cells isolated from the responding follicles and may have different apoptotic features. According to our results, it appears that the reduction in the number of granulosa cells per follicle in the older low-responder women, compared with young, normal responders, is a result of a non–apoptosis-mediated mechanism, leading to direct cellular atresia of granulosa cells in the older group. This interpretation is in accordance with that of other studies (24) showing that apoptosis can be the process responsible for atresia of quiescent (primordial and primary) follicles, whereas secondary follicles may escape the apoptotic process. Because ovarian tissue biopsies could not be offered for these patients, animal models may be the next research step.

We believe that further studies to explore the possible mechanisms of reproductive senescence by using microarray analyses may be the next step in searching for important genes that are involved in reproductive senescence.

REFERENCES

1. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002;8:141–54.
2. Abma JC, Chandra A, Mosher WD, Peterson LS, Piccinino LJ. Fertility, family planning, and women's health: new data from the 1995 National Survey of Family Growth. Vital Health Stat 1997;23:1–114.
3. Schwartz D, Mayaux MJ. Female fecundity as a function of age: results of artificial insemination in 2193 nulliparous women with azoospermic husbands. Federation CECOS. N Engl J Med 1982;306:404–6.
4. Shenfield F, Doyle P, Valentine A, Steele SJ, Tan SL. Effects of age, gravidity and male infertility status on cumulative conception rates following artificial insemination with cryopreserved donor semen: analysis of 2998 cycles of treatment in one centre over 10 years. Hum Reprod 1993;8:60–4.
5. van Blerkom J, Davis PW, Lee J. ATP content of human oocytes and developmental potential and outcome after in-vitro fertilization and embryo transfer. Hum Reprod 1995;10:415–24.
6. Seifer DB, DeJesus V, Hubbard K. Mitochondrial deletions in luteinized granulose cells as a function of age in women undergoing in vitro fertilization. Fertil Steril 2002;78:1046–8.
7. Hsieh RH, Tsai NM, Au HK, Chang SJ, Wei YH, Tzeng CR. Multiple rearrangements of mitochondrial DNA in unfertilized human oocytes. Fertil Steril 2002;77:1012–7.
8. Harman D. The biologic clock: the mitochondria? J Am Geriatr Soc 1972;20:145–7.
9. Fasouliotis SJ, Simon A, Laufer N. Evaluation and treatment of low responders in assisted reproductive technology: a challenge to meet. J Assist Reprod Genet 2000;17:357–73.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
11. Reers M, Smiley ST, Mottola-Hartshorn C, Chen A, Lin M, Chen LB. Mitochondrial membrane potential monitored by JC-1 dye. Methods Enzymol 1995;260:406–17.
12. Stocco DM, Clark BJ, Reinhart AJ. Elements involved in the regulation of the StAR gene. Mol Cell Endocrinol 2001;177:55–9.
13. Phy J, Stocco DM, Prien SD. Differences in steroidogenic acute regulatory protein expression from the luteinizing-granulosa cells of patients undergoing in vitro fertilization with embryo transfer: implications for cycle outcomes. Am J Obstet Gynecol 2002;186:872–5.
14. Depalo R, Nappi L, Loverro G, Bettocchi S, Caruso ML, Valentini AM, et al. Evidence of apoptosis in human primordial and primary follicles. Hum Reprod 2003;18:2678–82.
15. Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilization treatment. Lancet 1996;348:1402–6.
16. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 1987;65:1231–7.
17. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992;7:1342–6.

18. Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril 1999;72:837–44.
19. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod 2000;6: 1055–62.
20. Stouffer RL, Woodruff TK, Dahl KD, Hess DL, Mather JP, Molskness TA. Human recombinant activin A alters pituitary luteinizing hormone and follicle stimulating hormone secretion, follicular development and steroidogenesis during the menstrual cycle in rhesus monkeys. J Clin Endocrinol Metab 1993;77:241–8.
21. Klein NA, Battaglia DE, Woodruff TK, Badmanabhan V, Giudice LC, Brenner WJ, et al. Ovarian follicular concentrations of activin, follistatin, inhibin, insulin like growth factor I (IGF I), IGFII, IGF-binding protein-2(IGFBP-2), IGFBP-3 and vascular endothelial growth factor in spontaneous menstrual cycles of normal women of advanced reproductive age. J Clin Endocrinol Metab 2000;85:4520–5.
22. Sadraie SH, Saito H, Kaneko T, Saito T, Heroi M. Effects of aging on ovarian fecundity in terms of the incidence of apoptotic granulosa cells. J Assist Reprod Genet 2000;17:168–73.
23. Wei YH. Mitochondrial DNA alterations as ageing-associated molecular events. Mutat Res 1992;275:145–55.
24. Ebert KM, Liem H, Hecht NB. Mitochondrial DNA in the mouse preimplantation embryo. J Reprod Fertil 1988;82:145–9.

J Assist Reprod Genet (2006) 23:407–412
DOI 10.1007/s10815-006-9089-2

ANIMAL EXPERIMENTATION

Is estradiol mandatory for an adequate follicular and embryo development? A mouse model using aromatase inhibitor (anastrozole)

Muhammad Fatum · Yi Gyo · Prus Diana · Neri Laufer · Alex Simon

Received: 4 October 2006 / Accepted: 7 November 2006 / Published online: 6 December 2006

Abstract *Background:* Although high levels of estradiol are found in the follicular fluid, little is known about its necessity for adequate follicular growth, oocyte maturation and embryo development. Arimidex (anastrozole) is a potent aromatase inhibitor capable to induce an in-vivo milieu deprived of estradiol. This study uses a mouse model applying Arimidex to create an in-vivo system lacking of estradiol, in order to explore whether this gonadal steroid hormone is mandatory for folliculogenesis followed by normal fertilization and embryo development.

Methods: Experiment 1: Immature C57 Black female mice, aged 3–4 weeks were superovulated by 5 IU PMSG given intraperitoneally. A study group (9 mice) was concomitantly injected with 0.1 mg of Arimidex intraperitoneally given the morning day before PMSG, the morning day of PMSG injection and the following two days. The control group (8 mice) was similarly injected with normal saline. Estradiol (E2) and progesterone (P) serum levels were tested 48 hours after PMSG and the ovaries of each mouse blindly examined by a pathologist to evaluate follicular development. Experiment 2: 48 h after PMSG superovulation, hCG (7.5 IU) was injected intraperitoneally, followed by mating. The study group was treated with Arimidex 0.1 mg intraperitoneally daily from a day prior to PMSG injection to the day of sacrifice. The control group was treated similarly by normal saline. Forty-two hours after mating blood was withdrawn for E2 and P levels followed by tubal dissection. Embryos of 2–4 cells were cultured in-vitro and the development to the morula, blastocyst and hatching blastocyst stages were examined 24, 42, and 48 h later.

Results: Experiment 1: A significant reduction of E2 levels was achieved in the Arimidex group in comparison to control group (126.3 ± 104.8 and 1910 ± 960 pmol/L, respectively; $p < 0.0001$). Nevertheless, the two groups did not differ by the mean number of follicles (27 ± 9.5 and 30.4 ± 13.0) or the distribution for antral (65% and 68.4%) and pre-antral (35% and 31.6%) follicles, respectively. Experiment 2: The reduction of estradiol during follicular phase did not hamper follicular development, in-vivo fertilization and in-vitro embryo development. Similar rates of embryo development to the morula stage (90.6% and 86%), blastocyst stage (86% and 89%) and hatching blastocyst (81% and 78%) were achieved in the Arimidex group and the control group, respectively.

Conclusions: Adequate folliculogenesis is independent of estrogen but is conditioned on gonadotropin stimulation. Moreover, depletion of estradiol in the vicinity of the oocyte did not impair its developmental potential, including its fertilization and development into morulae, blastocysts and hatching blastocysts.

Keywords Anastrozole · Arimidex · Aromatase inhibitor · Embryo · Estradiol · Folliculogenesis · In-vitro fertilization · Mouse

Introduction

The development and maturation of ovarian follicles as well as the differentiation of granulosa cells are dependent on the effect of both gonadotropins, FSH and LH [1]. FSH receptors are exclusively present in granulosa cells, while the LH receptors are present in both thecal and granulosa

M. Fatum (✉) · Y. Gyo · P. Diana · N. Laufer · A. Simon
Department of Obstetrics and Gynecology, Hadassah University Hospital, Ein Kerem,
P.O. Box 12000, Jerusalem 91120, Israel
e-mail: fatum@md.huji.ac.il

cells. According to the "two cell, two gonadotropins theory" theca interna cells are stimulated by LH to produce aromatizable androgens. The androgens diffuse to granulosa cells and are converted to estrogens by aromatizing enzymes, the aromatase complex. Under FSH stimulation, granulosa cells undergo proliferation and differentiation, acquire LH receptors and aromatizing activity potential. Granulosa cells are the principal site for estrogen synthesis in the preovulatory follicle. Estrogens play an important intraovarian role in creating granulosa cells mitogenesis, the production of intracellular gap junctions, cAMP synthesis and further stimulation of steroidogenesis and gonadotropins receptors production.

In addition, there are several important extragonadal effects of the elevated estradiol levels during the follicular phase, i.e. the build-up of receptive endometrium, an important process preceding implantation, the production of the cervical mucus and pituitary priming for the mid-cycle LH surge.

At the follicle level, there is a growing body of evidence that estrogens have paracrine and autocrine functions at the cellular compartments of the developing follicle and the oocyte.

It is well established that estrogen alpha-receptor is present in the human oocyte [2]. Recently, with the isolation of the estrogen beta-receptor and its presence in the human granulosa cells, the old debate about the local effects and the importance of estradiol in the growing follicle was reopened.

The expression of both alpha and beta receptors in the mouse ovary was shown in several studies, revealing a distinct pattern of distribution of both estrogen receptors. Estrogen alpha-receptors are highly expressed in the interstitial/thecal compartment, whereas estrogen beta receptors expression is limited to granulosa cells of growing follicles [3, 4].

Although high levels of estradiol are found in the follicular fluid, little is known about its necessity for adequate follicular growth, oocyte maturation and embryo development.

Arimidex (Anastrozole) is a potent highly selective aromatase inhibitor lacking any other intrinsic hormonal activity that when used results in a selective decrease in estrogen synthesis.

In the present study, we apply Arimidex in a mouse model in order to study the importance of estradiol in folliculogenesis, oocyte maturation and in-vivo ovulation. In addition, the effects of hypoestrogenic environment on embryo development into morulae, blastocysts and hatching blastocysts are studied in-vitro.

Materials and methods

Animals

Immature C57Black female mice aged 3–4 weeks weighing 20–25 gm and mature 6–8 month old C57 Black male mice of a previously proved fertility were used. The animals were housed in air-conditioned quarters that were illuminated between 8:00 AM and 10:00 PM and pellet food and water were always available.

To induce superovulation, 5 IU of pregnant mare's serum gonadotropin (PMSG, Gestyl; Organon, Oss, The Netherlands) was injected intraperitoneally at 16.00 PM (day 1) followed by 7.5 IU of human chorionic gonadotropin (hCG, Sigma Chemical Co., St. Louis Missouri) given 48 hours later (day 3). After hCG administration, each female mouse was caged with a single male mouse and left overnight. Mating was confirmed by the presence of a vaginal plug on the following morning (day 4).

Medication

Arimidex (Anastrozole) an achiral benzyltriazole derivative is a potent, highly selective aromatase inhibitor with no intrinsic hormonal activities [5]. One tablet of the drug (1 mg) was crushed and grinded to a powder to be suspended in 2 ml of normal saline. As needed, 0.2 ml of the suspension (0.1 mg) was injected inraperitoneally to the female mice. The control groups were similarly injected by normal saline as appropriate.

Experiment 1: Is estradiol mandatory for normal folliculogenesis in-vivo?

Immature C57 Black female mice aged 3–4 weeks were superovulated as previously mentioned. The study group included nine mice. This group was concomitantly injected with 0.1 mg of Arimidex intraperitoneally given in the morning day (8 AM) before PMSG, the morning day of PMSG injection and for the following two days. The control group included eight mice and was similarly injected with 0.2 ml of normal saline.

Six to eight hours after the last Arimidex injection, the mice were anesthetized with Ketamin (Ketalar; Parke-Davis, UK) 100 mg/kg and Xylasine 2% veterinary (Vitamed LTD, Bat Yam, Israel) 5 mg/kg given intraperitoneally. Blood samples were withdrawn from the inferior vena cava for estradiol and progesterone levels. The ovaries of each mouse were dissected and sent for histological examination. Ovaries were fixed in formaldehyde solution embedded in paraffin wax and cut serially into 5 μm sections. These were stained with hematoxylin and eosin and both ovaries of each mouse were blindly examined by an experienced gynecopathologist to evaluate follicular development and search for any morphologic changes in its histologic appearance, if present.

Experiment 2: Is estrogen deprived microenvironment in-vivo affect embryo development in-vitro?

Mice were superovulated similarly by 5 IU PMSG given intraperitoneally.

Forty-eight hours later, 7.5 IU hCG was injected intraperitoneally followed by overnight mating. The study group (15 mice) was treated with Arimidex 0.1 mg injected intraperitoneally daily from a day prior to PMSG injection, in a similar manner to that scheduled in the first experiment, to the day of sacrifice. The control group (13 mice) was treated similarly by normal saline. Forty-two hours after mating (day 5), mice were anesthetized and blood was withdrawn for E2 and P levels followed by microscopic tubal dissection. Tubal dissection took place in human tubal fluid medium (HTF) supplemented with HEPES buffer and enriched with 0.4% bovine serum albumin (BSA, Sigma Chemicals Co., St. Louis, MO). Embryos of 2–4 cells were isolated and transferred for culture in P1 medium (Irvine Scientific, Santa Ana, CA, USA). Embryos were incubated at 37°C under a 5% CO_2, 5% O_2 and 90% N_2 environment and examined for the development of morulae after 24–26 h of culture. Morulae were transferred to blastocyst medium (Blastocyst medium, Ervine scientific, California, USA) and examined for the development to the blastocyst stage and hatching blastocyst after additional 18 and 24 h of incubation, respectively.

Statistics

Data were analyzed using the χ^2 test or Student's *t*-test as appropriate and *P* value of < 0.05 was considered as statistically significant. Statistical analyses were performed using a standard computer program of Microsoft Excel for Windows. Results are expressed in mean ± Standard deviation (SD).

Results

Experiment 1

As demonstrated in Table 1, a significant reduction of estradiol levels was achieved in the Arimidex group in comparison to the control group, 126.3 ± 104.8 pmol/L (mean ± SD) and 1910 ± 960 pmol/L, respectively ($P < 0.0001$). This indicates the efficacy of Arimidex suspension (0.1 mg intraperitoneally) used in this experiment to lower the follicular estradiol levels in the study group. Despite the significant difference in estradiol levels during the follicular phase, the two groups did not differ by the mean total number of developing follicles (27 ± 9.5 and 30.4 ± 13) nor by the distribution for antral (65% and 68.4%) and preantral (35% and 31.6%) follicles, for the study and control groups respectively (Table 1).

Table 1 Steroids serum levels and follicular distribution in the preovulatory period in the Arimidex and control groups

	Arimidex group ($n = 9$)	Control group ($n = 8$)	*P* value
Estradiol (pmol/L)	126.3 ± 104.8	1910 ± 960	< 0.0001
Progesterone (nmol/L)	10.2 ± 11.7	44.4 ± 19.4	< 0.0001
Total no. of follicles	27% ± 9.5	30.4 ± 13	NS
Antral (%)	65%	68.4%	NS
Preantral (%)	35%	31.6%	NS

Figure 1(A) and (B) show the histologic appearance of ovaries of the study group (A) and the control group (B). As demonstrated, both ovaries show similar number and distribution of growing follicles. No other morphologic changes, i.e., atretic or degenerative follicles, were noticed in both groups. Within the use of PMSG that posses also some hCG activity, luteinization may took place and explains the progesterone production during the follicular phase in both groups (Table 1).

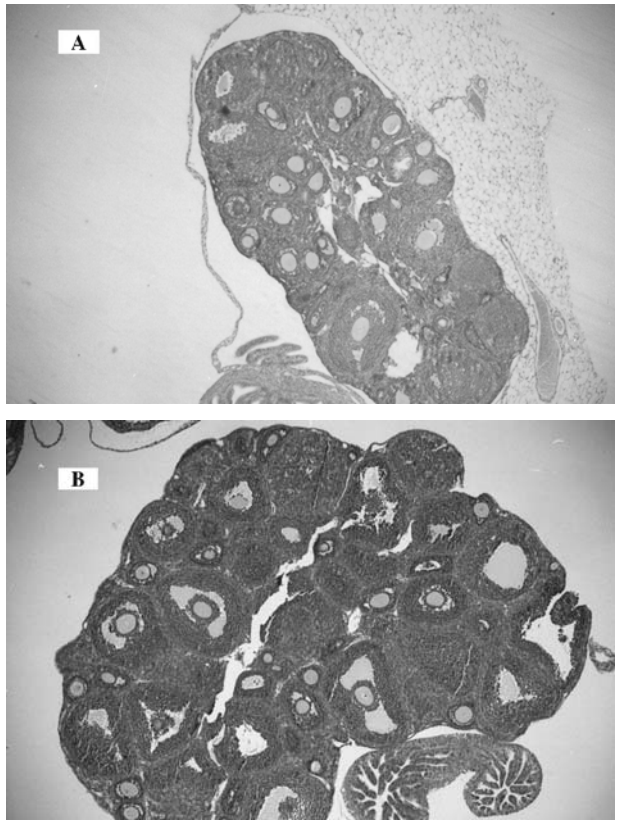


Fig. 1 The effect of Arimidex on ovarian histology. A. An ovary from Arimidex treated animal. B. An ovary from untreated animal. No difference in the histologic appearance with similar total number of follicles and the comparable preantral/antral distribution. Optical magnification X50

Table 2 Steroids serum levels during the luteal phase and embryo development in-vitro in the Arimidex and control groups

	Arimidex group ($n = 15$)	Control group ($n = 13$)	P value
Estradiol (pmol/L)	117.0 ± 32.8	200.5 ± 92.3	< 0.01
Progesterone (nmol/L)	207.9 ± 145.0	180.5 ± 110.9	NS
Total no. embryos 2–4 cells	360	335	
Morula stage (%)	326 (90.6%)	289 (86%)	NS
Blastocyst stage (%)	281 (86%)	256 (89%)	NS
Haching blastocyst (%)	228 (81%)	199 (78%)	NS

Experiment 2

Table 2 indicates the estradiol and progesterone serum level in blood withdrawn from the inferior vena cava just before tubal dissection. The estradiol level in the post-ovulatory phase is significantly higher in the control group in comparison to that in the Arimidex group. Its level, however, is significantly reduced in comparison to that in the pre-ovulatory phase. This reflects the luteinization that took place after the exposure to hCG and the shift to progesterone production in the corpora lutea.

The postovulatory progesterone levels were similarly elevated in both groups as compared to the pre-ovulatory levels, once again, indicating that luteinization took place in the study group despite the exposure to Arimidex.

In spite of the lower levels of estradiol induced by exposure to Arimidex during the follicular phase, ovulation took place followed by adequate mating and in-vivo embryo production. In addition, when the developmental potenial of these embryos was assessed in-vitro, similar rates of embryonic development into morulae (90.6% and 86%), blastocysts (86% and 89%) and hatching blastocysts (81% and 78%) were achieved in the study and control groups, respectively (Table 2).

Discussion

This study demonstrates that although a significant reduction of estradiol levels was achieved with Arimidex treatment during the follicular phase, it did not hamper follicular development, oocyte fertilization in-vivo and embryo development in-vitro. These results may indicate that, at least in mice, intaovarian estradiol plays no role in oocytes maturation or its developmental potential.

The high levels of estradiol during the follicular and the preovulatory phases are considered as the hallmark of the follicular phase. Estrogens are considered to play important roles and several pivotal activities at the ovarian follicle level. These include assisting granulosa cells proliferation and differentiation, augmentation of gonadotropin action, induction of gap junctions and acceleration of steroidogenesis. In addition, estrogens have several extragonadal effects: i.e. endometrial proliferation and cervical mucus biosynthesis.

Despite the high levels of estradiol present in the follicular fluid during the follicular phase, little is known about its necessity for adequate follicular growth, oocyte maturation and subsequent embryo development. During the last years, several studies have addressed to this important issues in order to elucidate the role of intraovarian estradiol. Nevertheless, among the different primate and non-primate animal models no uniform findings were achieved concerning this matter.

To date, studies addressing to this issue can be divided into three categories: I. Genetic mutations affecting enzymes participating in estradiol biosynthesis. II. Animal models with knock-out mutations in estrogen receptors. III. Aromatase inhibitors used in animal models.

Malproduction of estradiol

There have been several case reports showing multiple follicular development with the use of exogenous gonadotropin administration in women with congenital enzymatic defects on the estrogen biosynthesis pathway [6–8]. In these women despite normal development of follicles, ovum pick up and fertilization, no pregnancy was reported in those patients. These reports indicated that, in human, folliculogenesis as well as normal fertilization may take place unrelated to estradiol production. Although the number of cycles reported is low, embryo quality might be the crucial variable associated with the IVF failure.

In two women with mutations in the aromatase gene that results in total absence of aromatase activity, large ovarian cysts resembling the polycystic ovaries syndrome (PCOS) phenotype had been described indicating that the growth of antral follicles may occur despite the absence of intraovarian estrogen biosynthesis [9, 10].

Knock out mutations in estrogen receptors

It was shown [11] that a proportion of estrogen receptor knock-out (ER KO) mice can have ovarian folliculogenesis and normal ovulation with the development of what appeared to be a functional normal corpus luteum.

In addition, in the ovaries of mice lacking estrogen receptor β (ERβ-/-), follicles can be seen at all stages of development ranging from primordial to fully developed antral follicles [12]. However, when stimulated by gonadotropins, there was an increased number of antral follicles but low number of matured oocytes. Accordingly, fewer corpora lutea were observed resulting in a reduced fecundity. Taken together, these works challenged the view that estradiol has a mandatory role for folliculogenesis and ovulation in mice.

Aromatase inhibitors

There were several studies that utilized aromatase inhibitors, thus blocking the estrogen biosynthesis in animals. The results reported using different animal models were not consistent making it hard to draw a definite conclusion as to the effect of aromatase inhibitor on ovarian function. In rats, a negative correlation was observed between estrogen deficient environment and normal folliculogenesis leading to oocyte maturation [13]. Moreover, histological examination of the rat ovaries indicated a decrease in the number of the healthy follicles in the aromatase inhibitor treated group compared to controls.

In rhesus monkeys [14], the administration of aromatase inhibitor, 1,4,6-androstatrien-3,17-dione (ATD; Steraloids, Wilton, NH) during the late follicular phase of gonadotropin treated cycles resulted in 84% reduction of estradiol levels. However, there was no alteration in follicular diameters or the total number of folllicles per animal relative to control values. Nevertheless, completion of oocyte meiosis to metaphase II and the in-vitro fertilization of these oocytes were retarded in the treated group compared to control animals.

In other studies using Fadrozole (CGS 1694A) to block estrogen synthesis, it was shown that normal cyclical follicular maturation as well as normal hCG/LH induced ovulation were relatively unaffected in all animals studied (hamsters, rabbits, monkeys) other than rats [15]. These findings are in an agreement with our results achieved in a mouse model. We used Arimidex (anastrozol) suspension in a dose of 0.1 mg and were able to drastically lower serum estradiol levels during the preovulatory period. Nevertheless, follicular development, oocyte fertilization in-vivo and embryo development in-vitro were not affected. In addition, despite a significant reduction of estradiol levels, the histologic examination showed no difference in follicular development or in the distribution of preantral and antral follicles between the Arimidex treated group and the control group. Our findings corroborate with a recent report by Hu et al. [16] who utilized Arimidex to investigate the effects of reduced estrogen levels on in-vitro culture of preantral mouse follicles. Their findings indicated that in-vitro mouse follicles can develop normally under very low levels of estrogen and that a local androgen increase by a factor of 100 is not atretogenic. However, significantly less oocyte were fertilized in the group cultured in the presence of Arimidex, but, once fertilized, there was no difference in embryo development. The authors concluded that in mice a pronounced estrogenic environment is not essential for in-vitro folliculogenesis. In our study, however, we did not observe any negative correlation between Arimidex administration in-vivo and fertilization rates as was manifested by the same mean number of embryos collected from mice of the two groups.

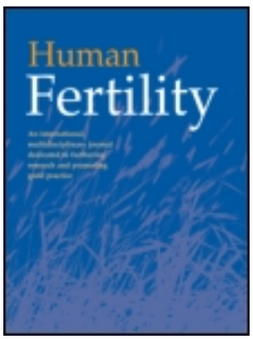
It should be mentioned that although estradiol levels were drastically reduced by Arimidex in our study, total reduction could not be achieved and thus, although low, estradiol levels might be sufficient enough to support normal folliculogenesis, adequate and healthy oocytes resulting in normal in-vivo fertilization and in-vitro embryo development. This assumption is supported by the report of Fisher et al. [17] who described the phenotype of aromatase knock-out mice to be infertile, with ovaries lacking corpora lutea. Similarly, Britt et al. [18] observed an age related degenerative changes in the ovaries of aromatase knock-out mice. Thus, by the age of one year there were no secondary or antral follicles and all primary follicles present were atretic. These findings suggest the possibility that total deprivation of estradiol may indeed interfere with ovarian function.

In summary, our study suggests that, in mice, adequate folliculogenesis is independent of estrogen but is conditioned on gonadotropin stimulation. Moreover, depletion of estradiol in the vicinity of the oocyte did not impair its developmental potential, including its in-vivo fertilization and in-vitro development into morulae, blastocysts and hatching blastocysts.

References

1. Hillier SG. Ovarian manipulation with pure gonadotropins. J Endocrinol 1990;127:1–4.
2. Wu TC, Wang L, Wan YJ. Detection of estrogen receptor messenger ribonucleic acid in human oocytes and cumulus-oocyte complexes using reverse transcriptase-polymerase chain reaction. Fertil Steril 1993;59:54–9.
3. Couse JF, Lindzey, Grandrian K, Gustafsson JA, Korach KS. Tissue distribution and quanitative analysis of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ) messenger ribonucleic acid in the wild wild type and ER-α knockout mouse. Endocrinollogy 1997;138:4613–21.
4. Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F, Giguere V. Cloning, chromosomal localization and function analysis of the murine estrogen receptor B. Mol Endocrinol 1997;11:353–65.
5. Dukes M, Edwards PN, Large M, Smith IK, Boyle T. The preclinical pharmacology of "Arimidex" (Anastrozole;ZD1033)-a potent, selective aromatase inhibitor. J Steroid Biochem Molec Biol 1996;58:439–45.
6. Meirow D, Schenker JG, Rosler A. Ovarian hyperstimulation syndrome with low estradiol in non-classical 17 alpha-hydroxylase 17, 20-lyase deficiency: what is the role of estrogens? Hum Reprod 1996;11:2119–21.
7. Pellicer A, Miro F, Sampaio M, Gomez E, Bonilla-Musoles FM. In-vitro fertilization as a diagnostic and therapeutic tool in a patient with 17, 20-desmolase deficiency. Fertil Steril 1991;55:970–5.
8. Rabinovici J, Blankstein J, Goldman B, Rudak E, Dor Y, Pariente C, Geier A, Lunenfeld B, Mashiach S. In-vitro fertilization and primary embryonic cleavage are possible in 17 alpha hydroxylase deficiency despite extremely low intrafollicular 17 beta-estradiol. J Cl Endocrinol Metabol 1989;68:693–7.

9. Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpso ER. A syndrome of female pseudohermaphordism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (p450arom). J Clin Endocrinol Metab 1994;78:1287–92.
10. Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Cl Endocrinol Metab 1995;80:3689–98.
11. Rosenfeld CS, Murray AA, Simmer G, Hufford MG, Smith MF, Spears N, Lubahn DB. Gonadotropin induction of ovulation and corpus loteum formation in young estrogen receptor-knockout mice. Biolo Reprod 2000;62:599–605.
12. Krege JH, Hodgin JB, Couse JF, Enmark E, Warne M, Mahler JF, Sar M, Korach KS, Gustafsson JA, Smithies O. Generation of reproductive phenotypes of mice lacking estrogen receptor-beta. Proc Natl Acad Sci USA 1998;95:15677–82.
13. Selvaraj N, Shetty G, Vijayalakshmi K, Bhatnagar AS, Moudgal NR. Effect of blocking oestrogen synthesis with a new generation aromatase inhibitor CGS 16949A on folliculr maturation induced by pregnant mare serum gonadotropin in the immature rat. J Endocrinol 1994;142:563–70.
14. Zelinski-Wooten MB, Hess DL, Baughman WL, Molskness TA, Wolf DP, Stouffer RL. Administrasion of an aromatase inhibitor during the late follicular phase of gonadotropin-treated cycles in rhesus monkeys: effects on follicular development, oocyte maturation, and subsequent luteal function. J Clin Endocrinol Metabol 1993;76:988–95.
15. Moudgal NR, Shetty G, Selvaraj N, Bhatnagar AS. Use of sepecific aromatase inhibitor for determining wether there is a role for oestrogen in follicle/oocyte maturation, ovulation and preimplantation embryo development. J Reprod Fertil Suppl 1996;50:69–81.
16. Hu, Y, Cortrrindt R, Smitz J. Effects of aromatase inhibition on in vitro follicle and oocyte development analyzed by early preantral mouse follicle culture. Mol Reprod Dev 2002;61:549–59.
17. Fisher CR, Graves KH, Parlow AF, Simpson ER. Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. Proc Natl Acad Sci USA 1998;95:6965–70.
18. Britt KL, Drummond AE, Cox VA, Dyson M, Wreford NG, Jones ME, Simpson ER, Findlay JK. An age related ovarian phenotype in mice with targeted disruption of the Cyp19 (aromatase) gene. Endocrinology 2000;141:2614–23.



Human Fertility

an international, multidisciplinary journal dedicated to furthering research and promoting good practice

ISSN: 1464-7273 (Print) 1742-8149 (Online) Journal homepage: https://www.tandfonline.com/loi/ihuf20

The case for aromatase inhibitors use in Oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in Breast Cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate

Muhammad Fatum, Enda McVeigh & Tim Child

To cite this article: Muhammad Fatum, Enda McVeigh & Tim Child (2013) The case for aromatase inhibitors use in Oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in Breast Cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate, Human Fertility, 16:4, 235-240, DOI: 10.3109/14647273.2013.800650

To link to this article: https://doi.org/10.3109/14647273.2013.800650

Human Fertility, 2013; 16(4): 235–240

ISSN 1464-7273 print/ISSN 1742-8149 online
DOI: 10.3109/14647273.2013.800650

informa
healthcare

SHORT REVIEWS

The case for aromatase inhibitors use in Oncofertility patients. Should aromatase inhibitors be combined with gonadotropin treatment in Breast Cancer patients undergoing ovarian stimulation for fertility preservation prior to chemotherapy? A debate

MUHAMMAD FATUM, ENDA MCVEIGH & TIM CHILD

Oxford Fertility Unit, Institute of Reproductive Sciences, Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, UK

Abstract

Breast cancer is one of the hormone-dependent cancers that may be adversely affected by elevated oestrogen or progesterone concentrations, particularly the endocrine active (hormone receptor positive) breast cancers.

Treatment for breast cancer patients aimed at fertility preservation, includes ovarian hyperstimulation, the harvest of oocytes, and subsequent cryopreservation of oocytes or embryos. Classically, gonadotrophins have been used effectively for ovulation induction, a treatment often accompanied by high blood oestrogen concentrations produced by the hyperstimulated granulosa cells. Despite the uncertainty which surrounds this issue and the lack of clear-cut clinical evidence, it is still of major concern that these ensuing high hormone levels might be associated with a high risk of recurrence of the cancer.

A growing number of clinical studies have strongly suggested the benefits of using aromatase inhibitors in infertility treatment, both as single agents or as adjuncts to FSH-containing ovulation induction regimes in reproductive medicine. Combining gonadotrophins with aromatase inhibitors would augment the stimulation effect, with a reduced increase in serum concentrations of estradiol. We propose to open a debate over the use of aromatase inhibitors in combination with FSH in ovulation induction treatment of breast cancer oncofertility patients.

As the safety of aromatase inhibitors such as letrozole has recently been demonstrated in several studies, and there is growing concern over the possible detrimental effects of high estradiol levels on breast cancer cells (at least in mouse models), the co-administration of letrozole in these patients would reduce both the high supraphysiologic serum levels of estradiol and the intratumoral in situ production of oestrogen. However, since it is unlikely that a well-founded evidence-based justification of this treatment will be formulated in the near future, based on well-designed prospective randomised controlled trials, we advocate a wider use of aromatase inhibitors in combination with gonadotrophins in breast cancer patients, especially those with hormone-receptor-positive tumours.

Keywords: *Cancer, cryopreservation, fertility preservation*

A growing number of clinical studies have strongly suggested the beneficial use of aromatase inhibitors in infertility treatment, both as single agents or as adjuncts to ovulation induction regimes using follicular stimulating hormone (FSH) in reproductive medicine (Mitwally & Casper, 2001; Mitwally & Casper, 2005). Aromatase inhibitors, mainly the third-generation agents, for example, letrozole, offer a seemingly effective, safe and low-cost option for ovulation induction in women with normal or increased levels of oestrogens such as in PCOS and in IVF patients who are poor responders to gonadotrophin stimulation (Al-Omari et al., 2004; Goswami et al., 2004).

Aromatase enzyme is a member of the Cytochrome P450 superfamily that catalyses the synthesis of oestrogens by converting C19 androgens to aromatic C18 oestrogenic steroids. It converts the aromatisable androgens, androstenedione and testosterone to oestrone and estradiol, respectively. Its activity can be demonstrated in several tissues, including the ovarian granulosa cells, adipose tissue, muscle, breast and liver.

Aromatase inhibitors have been introduced as an adjuvant hormonal treatment in breast cancer postmenopausal women after surgical and chemotherapeutic treatment. Breast cancer is one of the hormone-dependent cancers that may be adversely affected by elevated

Correspondence: Muhammad Fatum, NDOG, Level 3, Women's Centre, John Radcliffe Hospital, Oxford, Oxfordshire, OX3 9DU, United Kingdom. E-mail: Muhammad.Fatum@obs-gyn.ox.ac.uk

(Received 30 October 2012; revised 17 January 2013; accepted 4 March 2013)

oestrogen or progesterone concentrations, particularly the hormone (oestrogen and progesterone)-receptor-positive breast cancers.

With improvement in the diagnosis and treatment of early-stage breast cancer, an increased number of young women in their child-bearing years survive the disease.

Fertility treatment for cancer patients aimed at fertility preservation primarily includes ovarian hyperstimulation with the intention of superovulation and a high yield of oocytes, generally followed by either conventional in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI), leading to gamete/embryo cryopreservation. Classically, gonadotrophins have been used effectively for ovulation induction. However, the consequential endocrinological manifestation of this treatment is the accompanying high oestrogen blood concentrations produced by the granulosa cells. The potential detrimental effect of these iatrogenically induced supraphysiologic oestrogen concentrations is of major concern for patients and their oncologists, especially in women with hormone-receptor-positive tumours. Despite the uncertainty and the lack of clear-cut clinical evidence of this problem/issue, it is of paramount concern that the ensuing high hormone levels may be associated with a high risk of recurrence of the disease. On the other hand, clinicians might consider the length of time these women are likely to have had a cancer of the breast before it became clinically detectable. During that period of time, it would have been exposed to estradiol concentrations of the order of 1000 pmol/l at repeated intervals, and thus, one single further exposure might not be expected to have a measurable effect on overall prognosis.

In IVF cycles for infertile non-cancer patients, estradiol is required for the build-up of the endometrial lining in preparation for the implantation of the embryos. By contrast, in IVF cycles for cancer patients, which are undertaken for the purpose of obtaining gametes or embryos for cryopreservation, the endometrial lining build-up is unnecessary since the resulting gametes or embryos are cryopreserved for transfer long after the completion of the cancer therapy.

In recent years, the third-generation aromatase inhibitors (mainly anastrozole and letrozole) have been advocated as new ovulation-induction agents. These non-steroidal aromatase inhibitors are selective, highly potent, reversible inhibitors with no intrinsic steroidal activity. They are orally administered, easy to use, relatively inexpensive, and have minor adverse effects (Kafy & Tulandi, 2007; Al-Fadhli et al., 2006).

Letrozole is the most widely used aromatase inhibitor followed by anastrozole (Requena et al., 2008; Azim et al., 2007). These agents work by selectively inhibiting aromatisation, leading to diminished oestrogen production including estradiol, oestrone and oestrone sulphate. Consequently, there is release of the hypothalamic–pituitary axis from oestrogen-negative feedback. As a result, endogenous FSH secretion increases, stimulating folliculogenesis in the ovaries. The transient, relatively elevated intrafollicular androgens resulting from the diminished intraovarian aromatisation activity increase follicular sensitivity to FSH stimulation (Holzer et al., 2006; Mitwally et al., 2005). More recently, anastrozole has been shown not to suppress E_2 levels in women with breast cancer undergoing controlled ovarian stimulation sufficiently when compared to letrozole. In a prospective sequential cohort study investigating the potency of anastrozole compared with that of letrozole to suppress estradiol concentrations in breast cancer patients undergoing controlled ovarian hyperstimulation (COH), Azim et al. (2007) showed that anastrozole had minimal suppressive effect on rising estradiol concentrations during COH, even at five times the comparable dose of letrozole. Thus, breast cancer patients who underwent ovarian stimulation with anastrozole had significantly higher exposure to estradiol than those stimulated with letrozole. As a result, the study was terminated prematurely. In their conclusion, the authors cautioned against routine use of anastrozole in fertility preservation cycles.

Combining gonadotrophins with aromatase inhibitors would augment the stimulation effect of these drugs, with a lower increase in serum concentrations of estradiol. This decrease in serum concentration is hypothesised to be achieved by two mechanisms. First, using aromatase inhibitors enables the reduction of gonadotrophin dosage leading to reduced stimulation of granulosa cells and hence less estradiol production. A prospective study comparing the clinical outcomes between letrozole and clomifene citrate in gonadotropin-combined intrauterine insemination cycles (Jee et al., 2006) found that the concomitant use of Letrozole and gonadotrophins was associated with lower serum estradiol concentrations; however, the clinical pregnancy rate was similar in both groups.

Second, aromatase inhibitors cause a reduction in estradiol production by virtue of their direct downstream inhibitory effect on aromatase enzymes, thus inhibiting the aromatisation of target androgens in the granulosa cells.

We have reviewed the recent relevant literature in regard to the use of aromatase inhibitors in ovulation induction for cryopreservation in women with breast cancers. We propose that there should be a debate over the use of aromatase inhibitors in combination with FSH in ovulation induction treatment of breast cancer oncofertility patients. The safety of aromatase inhibitors has recently been shown in several studies, and there is growing concern over the possible detrimental effects of high estradiol concentrations in breast cancer cells of oncology patients. The co-administration of letrozole in these patients would reduce the high supraphysiologic serum concentrations of estradiol. Consequently, these potentially adverse, untoward effects would probably be reduced.

The importance of low estradiol levels

Every oncofertility expert can clearly articulate the dilemma in deciding to commence IVF cycles in

breast cancer patients because of the presumed risk of deleterious effects of elevated oestrogen concentrations on cancer cell proliferation. There have been no randomised controlled trials which have examined the relative efficacy, safety and effects (on progression-free survival rates) of the different ovulation induction regimens in breast cancer IVF patients, in particular the administration of aromatase inhibitors versus non-administration. We doubt whether there will be such studies in the near future since they may be viewed as unethical and of needless risk to the patients. In reality, this situation represents the intricate conundrum of taking clinical decisions when no evidence-based answers or policies are available. However, in these circumstances, lower-order/powered (non-RCT) and animal studies may provide indirect clues that help to answer this clinical problem, especially when it is clinically implausible and ethically debatable to implement such studies.

In their early study, Oktay et al. (2005) reported a prospective non-randomised trial aimed at developing safe ovarian stimulation protocols in breast cancer IVF patients wishing to preserve fertility before chemotherapy. Twenty-nine patients underwent 33 ovarian stimulation cycles either with tamoxifen 60 mg/day alone or in combination with low-dose FSH or letrozole 5 mg in combination with FSH. All resultant embryos were cryopreserved to preserve fertility. Recurrence rates were compared with those of 31 control patients who chose not to undergo IVF. The authors found that, compared to IVF patients treated with tamoxifen alone, IVF patients who had been treated with FSH and tamoxifen or FSH and letrozole had significantly greater numbers of follicles, mature oocytes and embryos. Peak estradiol concentrations were lower in patients treated with letrozole and FSH and with tamoxifen, compared with those in patients treated with tamoxifen and FSH. Recurrence rates after a mean follow-up of 554 ± 31 days was similar between IVF and control patients, and this was not affected by cancer stage. Of the three patients who had recurrence after IVF, two were in the tamoxifen group and one in the FSH group. There was no recurrence in the letrozole IVF group. The authors suggested that the letrozole protocol may be preferred because it resulted in lower peak estradiol concentrations. Nevertheless, the short follow-up, non-randomisation and low statistical power do not allow any clinically meaningful comparisons in survival between IVF and control patients to be made.

A subsequent retrospective age-matched control study of stages I–IIIA breast cancer patients, (Oktay et al., 2006) compared the efficacy of aromatase inhibitors plus gonadotrophin in breast cancer patients with a standard IVF protocol in non-cancer patients. Total oocytes, mature oocytes, fertilisation rate, the number of embryos and length of stimulation were similar between the two groups. Peak estradiol concentrations were significantly lower in the letrozole plus FSH group. In addition, there was a 44% reduction in gonadotrophin requirement in patients treated with letrozole and FSH for embryo/oocyte preservation compared with the controls. The authors concluded that ovarian stimulation with letrozole and FSH appeared to offer yields similar to standard protocols, reducing the amount of gonadotrophins required and hence providing a more cost-effective alternative for breast cancer patients. Considering the high cost of injectable gonadotropins, this could lead to significant savings for self-funding cancer patients who may already be stretched with costs associated with the stressful treatment of their primary disease (Oktay et al., 2006). In a later prospective non-randomised controlled study (Azim et al., 2008), a total of 215 women with breast cancer were evaluated prospectively for fertility preservation before adjuvant chemotherapy. The study group comprised 79 of these patients, who elected to undergo controlled ovarian stimulation using a combination of letrozole with gonadotrophins followed by oocyte collection for embryo or oocyte cryopreservation. The remaining 136 patients who underwent no fertility-preserving procedure served as controls. After a median follow-up of 23 months for the study group and 33 months for the control group, the authors reported no statistically significant survival difference between the two groups. Although this study is relatively small, non-randomised and the follow-up is limited, it does suggest that the use of letrozole and gonadotropins for controlled ovarian stimulation before embryo or oocyte cryopreservation is unlikely to result in a significant increase in recurrence rate at least in the short term, compared with those who do not undergo ovarian stimulation. The authors concluded that, in the meantime, based on the short-term follow-up of these patients, and until further follow-up to assess the impact of such therapy on the long-term recurrence or survival, the letrozole–FSH protocol is a viable option for oocyte or embryo cryopreservation (Azim et al., 2008; Partridge & Farber, 2008).

Animal models

Mice models of breast cancer have shown deleterious effects of high oestrogen concentrations on the cell proliferation rate of cancer cells. Thus, Platet et al. (2004) showed that oestrogens have an obvious role in cancer proliferation and invasion. Others (Brodie et al., 2006) used a tumour model with hormone-responsive (ER+) breast cancer cells stably transfected with the human aromatase gene. These cells serve as an autocrine source of oestrogen that stimulates the cells to form tumours when inoculated into ovariectomised, immunosuppressed mice. The resulting tumours were used to study strategies of treatment with aromatase inhibitors and anti-oestrogens. It was found that letrozole alone was better in this respect than the anti-oestrogen tamoxifen alone or in combination with letrozole. In addition, when tamoxifen treatment was no longer effective, tumour growth was significantly reduced in mice switched to letrozole treatment. One of the possible explanations

for the more beneficial effect of letrozole could be that it is not associated with weak agonist properties and therefore exerts a more complete reduction in oestrogenic effects than tamoxifen.

These data are of particular importance since results from the mouse model used in this study have been predictive of clinical outcome.

These basic-science studies, together with other studies on mice (Yue et al., 1994; Lu et al., 1998; Yue et al., 1999), provide supportive evidence for the relative risk to breast cancer cells' of exposure to elevated concentrations of estradiol and indirectly confirm the importance of adhering to tighter ovulation-induction regimes, with only minimal oestrogen production both systematically (by the ovaries) and in situ (intratumoral) production.

Human studies

Several studies have failed to show any beneficial effect of oestrogen exposure in recruiting cancer cells and increasing the proliferative fraction, on the chemotherapy response. One study (Fabian et al., 1994) was undertaken to test the effect of a high dose of oestrogen administered to patients with locally advanced ER-positive tumours and the subsequent flare effect on cancer cells, prior to chemotherapy. The hypothesis was that the oestrogen would increase chemotherapeutic efficacy in breast cancer by increasing the number of tumour cells in-cycle through hormonal recruitment prior to the initiation of chemotherapy. The high but dose of oestrogen administered to these patients reliably increased the tumour S-phase fraction and the proliferative index within 48 h. However, the response to Adriamycin-based chemotherapy did not appear to be markedly different from the locally advanced or metastatic disease patients. This study and others (Bontenbal et al., 2000; Paridaens et al., 1993) did not show any significant effect of administering oestrogen prior to chemotherapy, also indicating that deleterious effects from the lower oestrogen concentrations that may ensue using the aromatase inhibitors are unlikely.

Pregnancy rates with aromatase inhibitors

A concern has been raised that embryos derived from oestrogen deficient follicles as a result of aromatase inhibitor usage may not have the same developmental capacity as those obtained after treatment with conventional ovulation induction medications.

Indirect reassuring information has been obtained, initially from controlled ovarian hyperstimulation cycles that showed similar pregnancy rates in aromatase inhibitor and control groups. In a cohort study (Mitwally et al., 2005) that examined the early outcome of pregnancies achieved after treatment with letrozole compared with a control group that included pregnancies achieved spontaneously or after other ovarian stimulation protocols, treatment with letrozole for ovarian stimulation was associated with comparable rates of pregnancy and pregnancy loss (biochemical pregnancy and miscarriage). This was further confirmed in other pertinent studies (Jee et al., 2006; Atay et al., 2006; Aygen et al., 2007; Bayar et al., 2006; Badawy et al., 2009; Dehbashi et al., 2009; Zeinalzadeh et al., 2010) which reported similar pregnancy rates in the aromatase inhibitor and control groups.

A small number of non-randomised case-series studies have been reported on the use of aromatase inhibitors for IVF (Verpoest et al., 2006; Goswami et al., 2004; Garcia-Velasco et al., 2005; Schoolcraft et al., 2008), none of which found significantly different pregnancy rates in the aromatase inhibitor groups compared to the control groups.

In conclusion, the above-mentioned studies on the use of aromatase inhibitors in COH and IVF treatment cycles show no adverse impact on embryonic development when used as ovulation induction agents.

Safety profile of aromatase inhibitors in ovulation induction

Concerns have been raised about the safety profile of aromatase inhibitors. A small study presented as an Abstract to the American Society for Reproductive Medicine meeting in 2005 suggested that Letrozole could cause serious foetal anomalies (Biljan et al., 2005). The authors reported the outcome of 170 births, of which 20 were lost to follow-up. One hundred and fifty babies from 130 pregnancies born after the use of Letrozole were compared to a control group of 36,050 infants born from low-risk pregnant women. In this study, a malformation rate of 4.7% among the 150 babies was found comparing with a rate of 1.8% in the control group of normal conceptions. The incidence of cardiac and musculoskeletal system malformations was reported to be significantly higher in the letrozole-treated group. Several methodological problems of this report have been raised; the control group was younger (30.5 ± 1.2 years) than the letrozole-treated group (35.2 ± 4.7 years); only 110 women in the study group gave birth to singleton infants with the rest being multiple-foetal pregnancies which are known to be of higher risk of congenital malformations.

As a result of this report, the marketing company (Novartis) issued global warnings to healthcare professionals that the drug should only be used for its primary indication as a treatment for breast cancer therapy for postmenopausal women.

A large retrospective study (Tulandi et al., 2006) also disputed the results of the above-mentioned Abstract presentation. The authors reported the incidence of congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomifene citrate. They found 2.4% congenital and chromosomal malformations in the letrozole-treated group, and 4.8% in the clomifene citrate-treated group. The major malformation rate in the letrozole group was

1.2% and in the clomifene citrate group was 3%. The rate of all congenital cardiac anomalies was significantly lower in the letrozole-treated group (0.2%) compared to the clomifene citrate-treated group (1.8%). On the basis of these data, the concern that letrozole use for ovulation induction could be teratogenic is unfounded (Tulandi et al., 2006; Kafy & Tulandi, 2007; Requena et al., 2008).

Hu et al. (2002) had already shown that when oocytes were exposed to anastrozole there was no increase in spindle anomalies and the rate of development to blastocysts was similar to that of controls.

In another study (Luthra et al., 2003), aromatase-overexpressing mice were treated with high doses of letrozole for 6 weeks, and mated two weeks after the last letrozole dose. There was no difference in litter size, birthweight or the number of anomalies in the study group when compared with those in the controls.

In a study on mice, (Fatum et al., 2006), the administration of anastrozole and the consequent depletion of estradiol levels had no adverse effects on folliculogenesis or the number of oocytes produced after in vivo ovulation induction. When the resultant embryos were grown in vitro, there were no significant differences in embryo development to day 3 and subsequently to the morula and the blastocyst stages.

The short half-life of aromatase inhibitors and the administration of these drugs during the early follicular phase of the cycle leave a sufficient interval for complete washout to occur before fertilisation and implantation (Requena et al., 2008). The half-life of letrozole is 30–60 h, such that it should be cleared from the body by implantation (Casper and Mitwally, 2006).

Taken as a whole, these studies indicate that aromatase inhibitors are safe for usage in terms of folliculogenesis, in vitro embryo development and relative safety vis-à-vis teratogenic effects. Furthermore, in oncofertility patients where the resulting embryos are for cryopreservation, the teratogenity of aromatase inhibitors used in ovarian superovulation is largely irrelevant.

Conclusion

Despite the lack of well-designed RCT (randomised controlled trial) studies, we propose the use of aromatase inhibitors in combination with FSH in ovulation induction treatment of breast cancer oncofertility patients. The safety of aromatase inhibitors has been shown in several studies whereas there are possible detrimental effects of high estradiol concentrations in the breast cancer cells of oncology patients. The co-administration of letrozole and gonadotrophins in these patients would reduce both the high supraphysiologic serum concentrations of estradiol and intratumoral in situ oestrogen production. Consequently, potentially adverse effects of oestrogen production may be avoided.

We believe that, in the absence of clear-cut, contraindications (adverse or teratogenic effects) associated with the use of aromatase inhibitors, we should advocate a wider use of these agents in conjunction with gonadotrophins in breast cancer patients undergoing ovarian stimulation for fertility preservation, especially those with known hormone-receptor-positive tumours.

This is a classical clinical situation which poses a decision-making conundrum, where evidence-based medicine cannot be applied because of the lack of randomised controlled studies. Moreover, it is unlikely that in the near future there will be a well-founded evidence-based answer. Clinical judgement can be built on plausible biological effects and the acceptable safety of such a treatment policy. Clinical insight is integral to the clinical judgement and decision-making in this situation. We propose a policy which embraces clinical rationalisation and reasonable flexibility with open discussion with patients wherever there are no evidence-based studies or rigorous scientific data, the case in many areas of daily clinical practice. In other words, this discussion of the use of aromatase inhibitors in combination with FSH provides an excellent example of common clinical dilemma – the lack of randomised controlled studies. Our view is that this should not be considered an absolute contraindication that rules out a treatment.

Acknowledgement

We would like to thank Ms Tishy Bruce from Oxford Fertility Unit, for the linguistic revision of the manuscript.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

References

Atay, V.C., Muhcu, M., Cam, M., & Karateke, A. (2006). Comparison of letrozole and clomiphene citrate in women with polycystic ovaries undergoing ovarian stimulation. *Journal of International Medical Research*, 34, 73–76.

Aygen, E.M., Guzel, Z., Ozgun, T., Atakul, T., & Sahin, Y. (2007). The use of letrozole for ovulation induction in infertile women with polycystic ovarian syndrome. *Erciyes Tip Dergisi*, 29, 195–200.

Al-Fadhli, R., Sylvestre, C., Buckett, W., Tan, S.L., & Tulandi, T. (2006). A randomized trial of superovulation with two different doses of letrozole. *Fertility & Sterility*, 85, 161–164.

Al-Omari, W.R., Sulaiman, W.R., & Al-Hadithi, N. (2004). Comparisons of two aromatase inhibitors in women with Clomiphene-resistant polycystic ovary syndrome. *International Journal of Gynaecology and Obstetrics*, 85, 289–291.

Azim, A.A., Costantini-Ferrando, M., Lostritto, K., & Oktay, K. (2007). Relative potencies of anastrozole and letrozole to suppress estradiol in breast cancer patients undergoing ovarian stimulation before in vitro fertilization. *Journal of Clinical Endocrinology and Metabolism,* 92, 2197–2200.

Azim, A.A., Costantini-Ferrando, M., & Oktay, K, (2008). Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: A prospective controlled study. *Journal of Clinical Oncology*, 26, 2630–2635.

Badawy, A., Abdel Aal, I., & Abulatta, M. (2009). Clomiphene citrate or letrozole for ovulation induction in women with polycystic ovarian syndrome: a prospective randomized trial. *Fertility & Sterility*, 92, 849–852.

Bayar, U., Basaran, M., Kiran, S., Coskun, A., & Gezer, S. (2006). Use of an aromatase inhibitor in patients with polycystic ovary syndrome: a prospective randomized trial. *Fertility & Sterility*, 86, 1447–1451.

Biljan, M.M., Hemmings, R., & Brassard, N. (2005). The outcome of 150 babies following the treatment with letrozole or letrozole and gonadotropins. *Fertility & Sterility*, 84, S95.

Bontenbal, M., van Putten, W.L., Burghouts, J.T., Baggen, M.G., Ras, G.J., Stiegelis, W.F., Beudeker, M., Janssen, J.T., Braun, J.J., van der Linden, G.H., van der Velden, P.C., van Geel, A.N., Helle, P., Leisink, M., Foekens, J.A., Klijn, J.G. (2000). Value of estrogenic recruitment before chemotherapy: first randomized trial in primary breast cancer. *Journal of Clinical Oncology*, 18, 734–742.

Brodie, A., Sabnis, G., & Jelova, D. (2006). Aromatase and breast cancer. *The Journal of Steroid Biochemistry and Molecular Biology*, 102, 97–102.

Casper, R.F. & Mitwally, M.F. (2006). Aromatase inhibitors for ovulation induction. *The Journal of Clinical Endocrinology & Metabolism*, 91, 760–771.

Dehbashi, S., Dehbashi, S., Kazerooni, T., Robati, M., Alborzi, S., Parsanezhad, M.E., & Shadman, A. (2009).Comparison of the effects of letrozole and clomiphene citrate on ovulation and pregnancy rate in patients with polycystic ovary syndrome. *Iranian Journal of Medical Sciences*, 34, 23–28.

Fabian, C.J., Kimler, B.F., McKittrick, R., Park, C.H., Lin, F., Krishnan, L., Jewell, W.R., Osborne, C.K., Martino, S., Hutchins, L.F., Leong, L.A., & Green, S. (1994). Recruitment with high physiological doses of estradiol preceding chemotherapy: flow cytometric and therapeutic results in women with locally advanced breast cancers–a Southwest Oncology Group study. *Cancer Research*, 54, 5357–5362.

Fatum, M., Gyo, Y., Diana, P., Laufer, N., & Simon, A. (2006). Is estradiol mandatory for an adequate follicular and embryo development? A mouse model using aromatase inhibitor (anastrozole). *Journal of Assisted Reproduction and Genetics,* 23, 407–412.

Goswami, S.K., Das, T., Chattopadhyay, R., Sawhney, V., Kumar, J., Chaudhury, K., Chakravarty, B.N., & Kabir, S.N. (2004). A randomized single-blind controlled trial of letrozole as a low-cost IVF protocol in women with poor ovarian response: a preliminary report. *Human Reproduction*, 19, 2031–2035.

Garcia-Velasco, J.A., Moreno, L., Pacheco, A., Guillén, A., Duque, L., Requena, A., & Pellicer, A. (2005). The aromatase inhibitor letrozole increases the concentration of intraovarian androgens and improves in vitro fertilization outcome in low responder patients: a pilot study. *Fertility & Sterility*, 84, 82–87.

Holzer, H., Casper, R., & Tulandi, T. (2006). A new era in ovulation induction. *Fertility & Sterility*, 85, 277–284.

Hu, Y., Cortvrindt, R., & Smitz, J. (2002). Effects of aromatase inhibition on in vitro follicle and oocyte development analysed by early preantral mouse follicle culture. *Molecular Reproduction and Development,* 61, 549–559.

Jee, B.C., Ku, S.Y., Suh, C.S., Kim, K.C., Lee, W.D., & Kim, S.H. (2006). Use of Letrozole versus clomiphene citrate combined with gonadotropins in intrauterine insemination cycles: a pilot study. *Fertility & Sterility,* 85, 1774–1777.

Kafy, S. & Tulandi, T. (2007). New advances in ovulation induction. *Current Opinion in Obstetrics and Gynecology,* 19, 248–252.

Lu, Q., Yue, W., Wang, J., Liu, Y., Long, B., & Brodie, A. (1998). The effects of aromatase inhibitors and antiestrogens in the nude mouse model. *Breast Cancer Research and Treatment,* 50*,* 63–71.

Luthra, R., Kirma, N., Jones, J., & Tekmal, R.R. (2003). Use of letrozole as a chemopreventive agent in aromatase overexpressing transgenic mice. *Journal of Steroid Biohemistry and Molecular Biology,* 86*,* 461–467.

Mitwally, M.F. & Casper, R.F. (2001). Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. *Fertility & Sterility,* 75, 305–309.

Mitwally, M.F., Biljan, M.M., & Casper RF. (2005). Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. *American Journal of Obstetrics and Gynecology,* 192*,* 381–386.

Mitwally, M.F. & Casper, R.F. (2005). Single–dose administration of an aromatase inhibitor for ovarian stimulation. *Fertility & Sterility,* 83, 229–231.

Oktay, K., Buyuk, E., Libertella, N., Akar, M., & Rosenwaks, Z. (2005). Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and Letrozole for embryo cryopreservation. *Journal of Clinical Oncology,* 23, 4347–4353.

Oktay, K., Hourvitz, A., Sahin, G., Oktem, O., Safro, B., Cil, A., & Bang, H. (2006). Letrozole reduces estrogen and gonadotropin exposure in women with breast cancer undergoing ovarian stimulation before chemotherapy. *Journal of Clinical Endocrinology and Metabolism,* 91, 3885–3890.

Partridge, A.H. & Farber, D. (2008). Fertility preservation: A vital survivorship issue for young women with breast cancer. *Journal of Clinical Oncology,* 26, 2612–2613.

Paridaens, R., Heuson, J.C., Julien, J.P., Veyret, C., Van Zyl, J., Klijn, J.G., Sylvester, R., & Mignolet, F. (1993). Assessment of estrogenic recruitment before chemotherapy in advanced breast cancer: a double-blind randomized study. *Journal of Clinical Oncology,* 11, 1723–1728

Platet, N., Cathiard, A.M., Gleizes, M., & Garcia, M. (2004). Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Critical Reviews in Oncology/ Hematology,* 51, 55–67.

Requena, A., Herrero, J., Landeras, J., Navarro, E., Neyro, J.L., Salvador, C., Tur, R., Callejo, J., Checa, M.A., Farré, M., Espinós, J.J., Fábregues, F., & Graña-Barcia, M; Reproductive Endocrinology Interest Group of Spanish Society of Fertility. (2008). Use of letrozole in assisted reproduction: a systematic review and meta analysis. *Human Reproduction Update,* 14*,* 571–582.

Schoolcraft, W.B., Surrey, E.S., Minjarez, D.A., Stevens, J.M., & Gardner, D.K. (2008). Management of poor responders: can outcomes be improved with a novel gonadotropin-releasing hormone antagonist/letrozole protocol? *Fertility & Sterility,* 89, 152–156.

Tulandi, T., Martin, J., Al-Fadhli R., Kabli, N., Forman, R., Hitkari, J., Librach, C., Greenblatt, E., & Casper, R.F. (2006). Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertility & Sterility,* 89, 1761–1765.

Verpoest WM, Kolibianakis E, Papanikolaou E, Smitz J, Van Steirteghem A, Devroey, P. (2006). Aromatase inhibitors in ovarian stimulation for IVF/ICSI: a pilot study. *Reproductive Biomedicine Online,* 13*,* 166–172.

Yue, W., Zhou, D., Chen S., & Brodie, A. (1994). A new nude mouse model for postmenopausal breast cancer using MCF-7 cells transfected with the human aromatase gene. *Cancer Research,* 54*,* 5092–5095.

Yue, W., Santen, R.J., Wang, J.P., Hamilton, C.J., & Demers, L.M. (1999). Aromatase within the breast. *Endocrine-related Cancer,* 6, 157–164.

Zeinalzadeh, M., Basirat, Z., & Esmailpour, M. (2010). Efficacy of letrozole in ovulation induction compared to that of clomiphene citrate in patients with polycystic ovarian syndrome. *Journal of Reproductive Medicine,* 55, 36–40.

Ovarian Tissue Cryopreservation

7

Muhammad Fatum and Enda McVeigh

In recent years, cryopreservation of ovarian tissue in humans has emerged as a promising technique for fertility preservation in premenarchal children and adolescents, as well as an alternative in adult women prior to starting gonadotoxic chemo/radiotherapy. The prognosis and the survival rates of cancer patients have improved tremendously as a result of recent advances in cancer treatment – particularly in childhood cancers – and therefore attention is now being directed towards quality of life issues and the long-term gonadotoxic side effects of chemotherapy or radiotherapy. The incidence of ovarian failure is dependent on the agents used, the dose, and the age of the patient.

Currently, there are several modalities for fertility preservation and these are highly dependent on the age of the woman.

(i) Post-pubertal women requesting fertility preservation prior to chemotherapy or radiotherapy may be offered controlled ovarian stimulation for either oocyte cryopreservation or *in vitro fertilization* (IVF) for embryo cryopreservation. With IVF, harvested oocytes are fertilized with sperm produced by the husband, partner or donor, or are frozen as unfertilized gametes. This technology is in routine clinical use in IVF units throughout the world on a daily basis. Disadvantages include the need for hormonal stimulation for 2–4 weeks, which may defer the anticancer treatment, its relatively high cost, and the relatively limited number of embryos produced and stored from a single stimulation cycle. If there is a need to undergo immediate anticancer treatment, this is not a viable option.

(ii) Another option is obtaining immature oocytes (in non-stimulated cycles) for in-vitro maturation, subsequent embryo freezing or oocyte rapid vitrification.

(iii) Ovarian suppression prior to chemotherapy by GnRH analogues is of controversial significance and its use should be considered in well-designed experimental protocols.

(iv) Oophoropexy prior to radiotherapy (which includes an operative procedure, mainly laparoscopy, to move ovaries out of the pelvic radiotherapy field) is an appropriate treatment for only a very few well-defined group of patients.

(v) Ovarian tissue cryopreservation is a fertility preservation approach that can usually be performed immediately and without hormonal stimulation. It is the only available fertility preservation approach that can be offered to premenarcheal/prepubertal girls and to adult women undergoing aggressive

M. Fatum, MD (✉) • E. McVeigh, FRCOG
Nuffield Department of Obstetrics and Gynaecology,
Institute of Reproductive Sciences,
University of Oxford, Oxford, UK
e-mail: muhammad.fatum@obs-gyn.ox.ac.uk;
enda.mcveigh@obs-gyn.ox.ac

M. Metwally, T.-C. Li (eds.), *Reproductive Surgery in Assisted Conception*,
DOI 10.1007/978-1-4471-4953-8_7, © Springer-Verlag London 2015

chemotherapy/radiotherapy with a high likelihood of gonadotoxicity who cannot defer the anticancer treatment to undergoing IVF cycle. In this modality, functional ovarian cortical tissue is surgically excised from the ovary and frozen. Laparoscopy is usually performed to harvest ovarian tissue, followed by tissue processing and cryopreservation. Following recovery from the cancer treatment and when the patient is ready to proceed with fertility treatment, the ovarian tissue is thawed out and autotransplanted.

Laparoscopic Ovarian Biopsy for Cryopreservation

Indications

The American Society of Clinical Oncology (ASCO) has published clinical practice guidelines [1] to aid in gonadotoxicity estimation and define indications of fertility preservation in the different cancer diagnoses and treatment protocols. Similar guidelines have been published by International Society of Fertility Preservation (ISFP) [2]. These guidelines provide a clear basis for good clinical practice.

Several factors should be taken into consideration when ovarian tissue cryopreservation is discussed with these patients: patient's age, patient's oncologic prognosis and quality of life, fertility and obstetrical history, availability and affordability of other fertility preservation technologies, explaining and understanding the meaning of success as per the most up-to-date data in the literature and counselling for the psycho-social perspectives.

Every programme should have its own policy and inclusion and exclusion criteria for ovarian tissue cryopreservation. In our programme, we include females aged not more than 39 years of age and no less than 12 months old, with reasonable (>50 %) chance of surviving 5 years and a high (>50 %) risk of having ovarian function destroyed by therapy. Another important inclusion criterion is that the patient should be fit enough to undergo general anaesthesia and surgery. Table 7.1 summarizes the paediatric groups included in our ovarian tissue cryopreservation programme.

Table 7.1 major paediatric patients groups included in the ovarian tissue cryopreservation programme in Oxford

Whole abdomen and pelvic radiation greater or equal to 15 Gy in pre pubertal or 10 Gy in post pubertal girls especially in combination with any alkylating agents
Craniospinal radiation
Total Body irradiation as part of conditioning for bone marrow transplant
High dose chemotherapy:
Cyclophosphamide 120–200 mg/kg as conditioning for BMT
Cyclophosphamide >5 g/m^2
Cisplatin
Ifosfamide >60 mg/m^2
Melphalan 140–210 mg/m^2
Busulphan 8–16 mg/kg
Thiotepa
Procarbazine
BCNU/CCNU

As programs progress and more clinical data becomes available, we believe that ovarian tissue cryopreservation will be increasingly offered to non-malignant diseases such as females at risk of premature ovarian failure: i.e. Turner syndrome, family history of premature ovarian insufficiency, benign autoimmune diseases requiring gonadotoxic chemotherapy or patients needing bone marrow transplantation for benign haematological diseases such as sickle cell anaemia and thalassaemia major.

Following referral it is mandatory to have a thorough pre-treatment consultation by a multidisciplinary team that specializes in fertility preservation. This team should include an oncologist, a reproductive endocrinologist, a surgeon, specialized nurse and a paediatrician and social worker as required. The cancer diagnosis, the chemotherapeutic agents and their doses, radiotherapy field and scattered radiation doses, as well as the patient's age should to be taken into consideration before deciding on the need and the extent for the ovarian tissue cryopreservation.

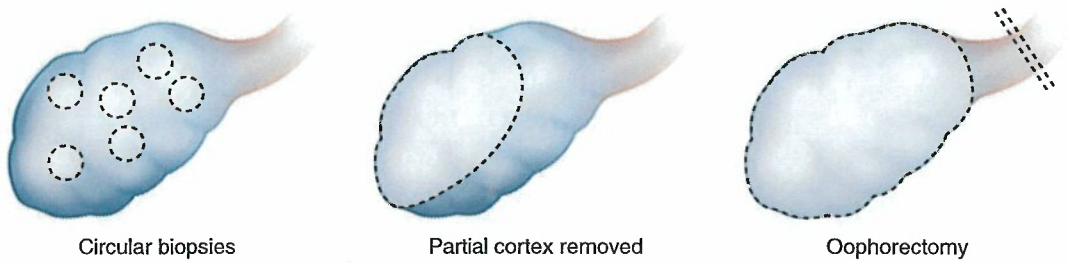


Fig. 7.1 Shows the different ovarian tissue harvesting options: biopsy, partial ovarian cortex resection, unilateral oophorectomy. The amount of the ovarian cortical tissue harvested is tailored to the gonadotoxicity degree of the anti-cancer treatment and the ovarian size

Preoperative Workup

Preoperative workup of the patient is performed to exclude ovarian pathology and possible pelvic metastases (e.g. gynecologic ultrasonography, CT scans or MRI) [3]. During laparoscopy, a meticulous inspection of both ovaries is performed to look for malignancy [4–6]. Ovarian activity is measured by either measuring the antral follicular count by ultrasound scan or testing patient's blood for ovarian reserve markers, i.e. Anti Mullerian Hormone (AMH) or Follicle Stimulating hormone (FSH).

Ovarian cortical tissue harvesting is commonly performed as a laparoscopic procedure under general anaesthesia. Three or four entry puncture sites are used: 12 mm intraumbilical trocar , two 5 mm trocars in the left and right iliac fossae. A fourth 12 mm suprapubic trocar can be used if oophorectomy is performed to allow atraumatic ovarian removal with an endobag. If open surgery is planned for the patient, ovarian biopsies should be taken at the time of the surgery. Other procedures requiring a general anaesthetic can be performed at the same time such insertion of Hickman catheter prior to starting chemotherapy.

Ovarian biopsies are taken either with laparoscopic forceps or scissors. It is important to minimize as much as possible the use of diathermy so as not to damage the ovarian tissue. If bleeding does occur, this is best controlled with small, precise mirco-bipolar diathermy. Care should also be taken to minimize trauma to the ovarian tissue and the prompt removal of the tissue to the laboratory team.

The ovarian cortical tissue size harvested or number of biopsies taken is dependent on the estimated gonadotoxic effects of the planned chemotherapy and/or radiotherapy regimen, varying from 20 biopsies (10 biopsies from each ovary) or 2 cortical strips measuring 15 mm long and 5 mm wide taken from each ovary. When a high-risk chemotherapy or radiotherapy is planned and risk of premature ovarian failure is high (60–80 %), oophorectomy should be considered (Fig. 7.1). Ovarian tissue cryopreservation can also be combined with intraoperative retrieval of immature oocytes from the contralateral ovary in cases of oophorectomy or from both ovaries if only biopsies are taken. Retrieved oocytes are then subjected to in vitro maturation and cryopreservation of either mature oocytes or embryos.

Processing the Ovarian Tissue

Preparation of the ovarian tissue, cryopreservation and thawing must ensure follicle viability and integrity of tissue compartments and cell-to cell contacts [7]. Two well-established methods can be used: rapid vitrification and slow freezing of finely dissected thin slices of ovarian cortex tissue [8–11]. Both freezing methods are acceptable with good survival rates and comparable tissue viability and morphology integrity rates. Several recent reports have shown improved outcomes after vitrification of ovarian tissue as compared to slow freeze technique in preserving granulosa cells and ovarian stroma [12, 13]. However, until clinical outcome

data show improved outcome of the vitrification protocol, we think the slow freeze protocol should be adopted. In the upcoming sections the slow freeze protocol used in our Unit will be described.

Ovarian Dissection

The cooled ovary should be transported from the operating theatre to the laboratory within 30 min of procurement and then tissue processing is immediately commenced within a clean facility in the laboratory. Antral follicles are aspirated at the beginning and fluid is carefully assessed for the presence of oocytes. In addition, the remaining media left after ovarian tissue dissection is ideally given to embryologists at the end of the procedure to check for potential immature oocytes. If immature oocytes are found then they undergo in vitro maturation (IVM) and the resultant mature eggs are vitrified. The ovary is immersed in a petri dish in cooled Leibovitz 15 medium and is bi-valved using sterile scalpel and forceps or tweezers (Fig. 7.2). The inner medulla tissue is dissected away leaving the thin outer layer of the cortex that contains the majority of follicles [9, 14]. Our practice is to have two staff working together to minimise damage to tissue by reducing the overall processing time and reducing the length of ischaemia before starting the freezing procedure. The cortical tissue slices are all trimmed to within a standard thickness of 1–2 mm to ensure that cryoprotectants adequately diffuse into the ovarian tissue during the cryoprotection procedure prior to freezing. In addition, after autografting, thin slice grafts are more likely to survive the post transplantation ischemic stage,

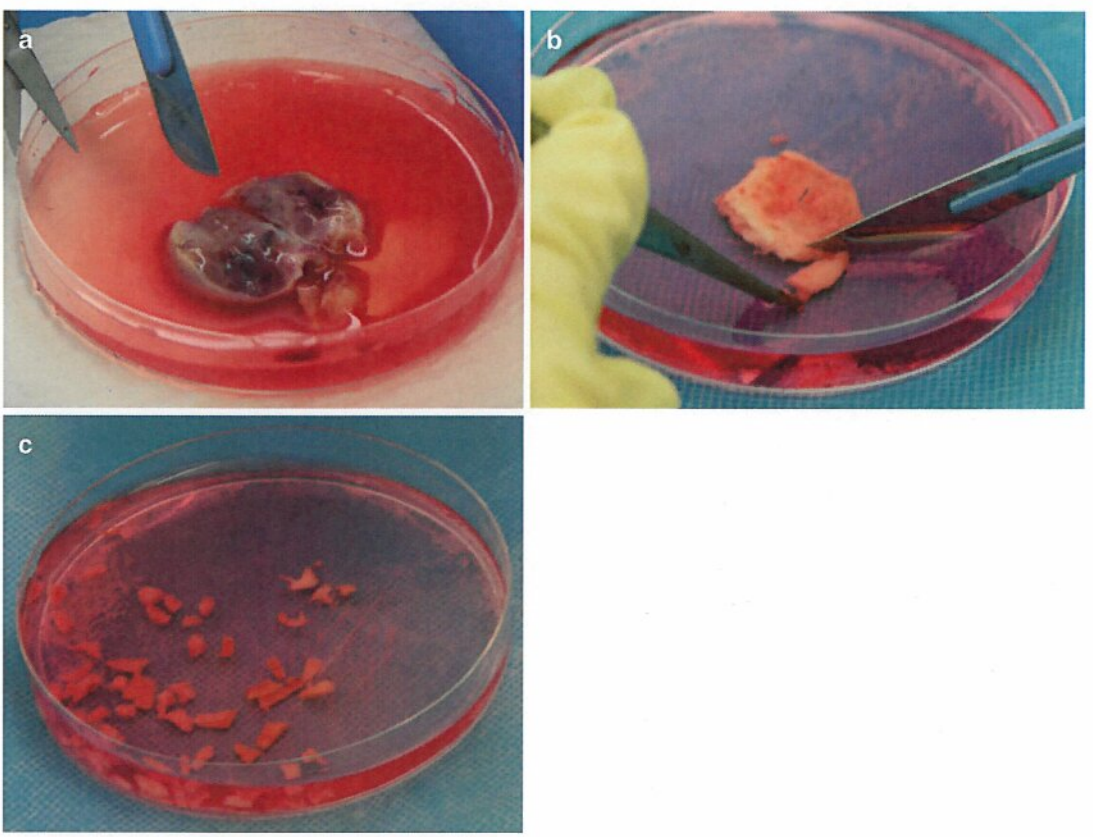


Fig. 7.2 Ovarian tissue processing in the laboratory as demonstrated for whole ovary removal. (**a**) The ovary is bi-valved. (**b**) The medulla is cut and carefully removed and the 1 mm thickness outermost cortex is achieved. (**c**) The ovarian cortex slices before freezing

by more efficient simple diffusion before neovascularisation and perfusion are adequately established [10, 15, 16]. The slices are then cut in small pieces of 1 mm width and 5 mm length. Other groups reported variable sizes of the slices. There seems to be no difference in results in the different sizes: the most critical factor is the slice thinness. Slices are then placed in fresh Leibovitz medium until cryoprotection.

Quality Assessment (After Dissection)

A standard practice in centres performing ovarian tissue cryopreservation is to perform routine tests to ensure primordial follicles are present in the ovarian tissue slices and that they are morphologically normal. At this stage, these tests primarily include light microscopy examination to assess the percentage of primordial follicles found in fresh (or pre-processed) ovarian tissue slices [10, 17–20]. Hence, a specimen should be taken during dissection from the ovarian cortex and sent off for histopathological evaluation by light microscopy (×400 magnification) after fixation in Bouin's solution and using haematoxylin/eosin staining to check for the presence of follicles and morphology of the tissue. Samples are also tested using standard trypan blue viability staining methodology. Another specimen is placed in 4 % formaldehyde and sent for pathological evaluation for the presence of malignant cells, prior to cryopreservation. Other samples are also taken and stored separately for post-thaw testing before reimplantation in the future. All histological tests are ideally performed by the same specialised consultant pathologist using the same protocols.

Cryoprotection

To minimise damage due to normally lethal intracellular ice crystal formation and build-up of extracellular salt concentration, the tissue is quickly transferred after dissection into test tubes containing 8 ml of pre-cooled cryoprotectant medium and labelled. We use a mixture containing 1.5 M penetrating cryoprotectant medium in order to dehydrate cells and increase solute concentration around the cells. We use the ethylene glycol as an effective cryoprotectant for ovarian tissue as was shown in several works [10, 11, 15]. The non-penetrating sugar, sucrose (0.1 M) is also added to the medium since it is known to act as an osmotic buffer and is an added precaution against excessive osmotic swelling during cryoprotectant removal and thus minimises freezing injury [21]. The tissue is incubated on a shaking plate at 2–8 °C for exactly one hour. Three to five tissue slices are then quickly transferred to cryovials with 1 ml of cooled cryoprotectant. The permeation of isolated cells with cryoprotectants is relatively fast, but diffusion through multicellular tissues such as ovarian cortical tissue slices is much slower i.e. exposure to cryoprotectant is prolonged to ensure adequate concentrations in the centre of the tissue. However, this has to be balanced with the fact that cells on the tissue surface may be at risk of excessive toxicity. Cryoprotection time periods should therefore be carefully standardised and strictly adhered to.

Freezing

The freezing protocol of slow freezing has long been well established and optimised designs for ovarian tissue cryopreservation are very similar [3, 10, 14, 15]. As the temperature is cooled, ice crystals growth is initiated by ice crystal nucleation induction (i.e. 'seeding'). As the crystals grow the water in the solution is turned to solid state, increasing the solute concentration and this draws water out of the cells [22]. More water can be incorporated into ice at lower temperatures but the rate at which water can leave a cell also falls as temperature is lowered. The effectiveness therefore depends upon equilibrium between the rate at which water can leave a cell and the rate at which it is converted into ice. The rates used by our programme classically involve an initial cooling rate of approximately –2 °C/min down to –9 °C with then a hold for 15 min to perform 'seeding'. We achieve this by using long handled metal forceps dipped in liquid nitrogen and gripping the cryovials at the meniscus. This is followed by a 5 min soaking hold period. Then a slower rate of cooling –0.3 °C/min is programmed to reduce temperature down to –40 °C. The final stage of the freezing program is a faster cooling stage of –10 °C/min down to –140 °C. All tissue is frozen in the

same programmable Sylab freezer (Ice Cube) using the same program.

Storage

The cryovials containing the tissue are then sealed inside an additional sterile cryogenic bag, which is labeled and placed in a protective cardboard box and then transferred to liquid nitrogen storage into the predetermined quarantine rack position. Many establishments then plunge their ovarian tissue directly into liquid nitrogen, however, we prefer to store tissue in vapour phase nitrogen (at approximately −170 °C) rather than in liquid nitrogen (at −196 °C) in order to minimise risk of cross-contamination of tissue packaging from bacteria or viruses surviving in the nitrogen tank [23]. This does not affect the quality of tissue storage, since below −130 °C, the glass transition temperature of water, no biological or physical changes take place and therefore below this temperature tissue may be safely stored [22].

Thawing Out

Thawing techniques are a standard amongst the different ovarian processing units [10]. The rapid thawing process is performed within the clean room facility in the laboratory whilst the patient is in operating theatre in order to minimise ischaemic time. The relevant cryovials are removed from the nitrogen storage freezer and held in air for 30 s. The cryovial is then placed in a water bath at 30 °C for 3 min. The cryovial is then transferred to the cleanroom microbiological safety cabinet for three-stage serial dilution of the ethylene glycol, each stage taking 5 min and performed on a shaker at room temperature. The ovarian slices then remain in a test tube with a final mixture of 1 ml sucrose, 1 ml serum substitute supplement and 8 ml Leibovitz medium. The same protocol is also used for all specimens used for testing.

Post Thawing

Quality Assessment

Specimens of the ovarian cortex are thawed out approximately 1 month prior to the planned auto-transplantation using the same protocol as for tissue slices for clinical use. They are then sent off for the same histopathologic evaluation of the presence, viability and morphology of follicles as was performed following dissection, involving the same histological techniques and trypan blue staining [17–20]. Published studies consistently show there is only minimal difference between results for fresh and for frozen-thawed tissue [10, 17]. The number of follicles counted, however, will depend upon age of donor [17, 22] and whether any previous treatment of chemo- or radiotherapy has been given prior to procurement of ovarian tissue.

Screening for Micrometastases

The major concern with ovarian tissue banking is the possibility of re-seeding a tumour, harboured within the ovarian slices, after auto-grafting the frozen thawed tissue slices. Tissue samples are therefore carefully screened for the presence of malignant cells (micrometastases) by histology and specific immunohistochemical or molecular tests such as polymerase chain reaction (PCR) testing and real-time PCR to detect molecular markers that would indicate presence of cancer cells [3, 6, 24]. The various tumours are categorised into three groups according to their risk of ovarian micro-metastasis [6, 25] and this should be taken into account upon the initial consultation as part of informed consent. Testing should be performed approximately 1 month prior to planned transplantation using the up-to-date licensed methods available at the time of re-implantation, thus taking advantage of the fact that diagnostic methods for detecting minimal residual disease are likely to be improving with time. When tissue shows evidence of malignant cells (i.e. any positive test result) , the patient is advised against future auto-transplantation of ovarian cortical tissue slices. Instead, in the future these patients may benefit from later advances and possible breakthroughs in the field of In Vitro Maturation (IVM) of ovarian tissue, in vitro maturation of follicles or isolated follicle transplantation, which probably do not pose any risk to transmission of tumour cells [26]. A combined

immature oocyte recovery for IVM, in addition to the ovarian tissue cryopreservation may be of critical importance in these patients.

Auto-transplantation

Auto-grafting of the tissue is done when the patient is in remission and old enough and ready to start a family. The tissue is commonly transplanted in the orthotopic position (i.e. into its natural site such as the ovarian hilum or medulla or nearby structures such as the broad ligament or the pelvic sidewall). This has been shown to offer the potential for spontaneous pregnancy without the need for primary and immediate resort to IVF [10, 15, 27–29]. Alternatively, heterotopic transplantation (e.g. non-native ectopic location such as the arm or abdomen) has certain advantages [9, 30, 31] such as easier follicular monitoring and egg retrieval for IVF as well as a closer monitoring for cancer recurrence following auto-grafting. Nevertheless, orthotopic transplantation has been shown to result in less follicle loss and more effective revascularisation and is believed to be more effective [31]. Orthotopic autotransplantation can be performed either by laparotomy or laparoscopy. Some advocate [32] performing it in two stages: in the first stage laparoscopy is performed 7 days before the re-implantation procedure, aiming at creating a peritoneal window at the ovarian hilum, broad ligament or the pelvic sidewall. The goal of this procedure is to induce neovascularization and the formation of granulation tissue in the area where the ovarian slices are to be re-implanted. It is believed that by this neoangiogenesis, the ischemic phase after the transplantation is shorter. A second stage laparoscopy follows after about a week during which the ovarian slices are transplanted into the peritoneal furrow. The slices can either be sutured to the ovarian medulla or left unsutured into the peritoneal window. Pregnancies were reported after auto-grafting of varying sizes of ovarian slices [33] as well as after micro-organ transplantation of thinned ovarian slices [15].

Outcome of Frozen-Thawed Ovarian Tissue Autotransplantation

To date more than 30 pregnancies have been reported after autotransplantation of ovarian cortical tissue slices mainly after orthotopic but also after heterotopic reimplantation [33]. From reviewing the accumulative experience as reflected in the different case series and case reports published so far, it is noted that it takes 3.5–6.5 months on average after autografting of the ovarian tissue until signs of endocrinologic activity (a rise in estradiol levels and a drop in FSH levels) and folliculogenesis (as observed in ultrasound scanning) are detected. Restoration of ovarian activity is observed in 93 % of patients after autografting. The post-transplantation pregnancies reported thus far included natural conceptions, ovulation induction cycles and IVF cycles [33]. The average duration of ovarian function after transplantation is 4–5 years [33, 34] and is mainly dependent on the ovarian reserve before the harvesting and the absence of chemotherapy before cryopreservation. These promising results strongly suggest that ovarian tissue cryopreservation is a viable fertility preservation option that should be part of any oncofertility programmes.

Challenges with Ovarian Tissue Preservation

Restoration of ovarian function with hormone production and follicular growth has been observed after transplantation of frozen–thawed ovarian tissue [30, 35–38], and more pregnancies and livebirths are increasingly reported [32–34]. However, efforts are still needed to develop and optimize harvesting and grafting procedures to increase the success rates. A large percentage of the grafted follicles are lost as a result of post-grafting ischemia before efficient neoangiogenesis networks are established. In addition, reperfusion-induced damage might contribute to this loss [39–41]. It is estimated that more than 50 % of primordial follicles are lost following

ovarian transplantation due to ischemia. It takes 4–5 days for neoangiogenesis to result in adequate perfusion and reoxygenation of the grafts. This delay in graft perfusion and reoxygenation is believed not only to lead to follicle loss, but also to dysregulated communication and asynchrony between granulosa cells and oocytes [42–44]. Basic biomedical research studying revascularisation of the grafts may prove important in improving the technique by reducing the ischemia period until the reconstitution of efficient vascularization and perfusion. This can possibly be done by delivery of angiogenic factors like VEGF or by preparing the vascular bed prior to autografting procedure [33, 45–47].

A second important issue is the burnout effect, in which a relatively large amount of follicles are recruited and over activated as a result of reduced inhibitory mechanisms, such as those mediated by AMH. It has been shown that recovered grafts have higher ratios of growing to total follicles and higher levels of proliferation staining than non-transplanted control tissue [48], and this phenomenon was demonstrated to a greater degree in thinner grafts. It is hypothesized that disruption of the ovarian homeostasis as a result of the removal of follicles from their normal physiological environment together with the absence of larger follicles in the auto-grafted ovarian cortical strips result in a decrease in the production and secretion of the inhibitory signalling factor AMH , consequently leading to follicle activation and burnout [48]. More basic biomedical studies are needed to thoroughly understand the molecular basis and mechanisms involved in the burnout phenomenon and its relative role in graft function and lifespan.

Thirdly, some studies have demonstrated a risk of possibly transmitting malignant cells present in the cryopreserved tissue back to a patient's body [3, 49]. Hence, one of the most important concerns of ovarian tissue cryopreservation is the re-seeding and relapse of a tumour. The ovarian tissue micrometastases risk of the different types of cancers and their stages is classified into three groups: (a) the low to no risk group – e.g. Non-Hodgkin's lymphoma, breast cancer stage I-III, Ewing's sarcoma (b) the moderate risk group– e.g. Breast cancer stage IV, infiltrative lobular colon cancer, upper GI system malignancies, and (c) the high risk group– e.g. Leukemia, Burkitt's lymphoma and Neuroblastoma.

The risk that the post-thaw tissue from these patients may re-seed malignant cells of the primary tumour into the cured patient as a result of transplantation, should be ruled out prior to the autotransplantation as discussed above.

The in-vitro culturing and growth of immature follicles derived from ovarian slices collected prior to commencement of cancer treatment is an important direction for research to avoid the need for autografting in patients at high risk of cancer reseeding. The purpose of the in-vitro human follicle growth system is to mimic the in vivo process by sequentially providing follicles with the necessary levels of the important growth factors and hormones at the right time, which can allow follicular growth and oocyte maturation through maintaining the essential connections between the two components of the follicle compartment [50, 51]. Successful in-vitro culturing and maturation of the ovarian tissue or isolated follicles to produce mature follicles and competent oocytes that could be subsequently fertilized to produce embryos would provide a safe fertility preservation option. Currently this field is the target of intensive research and in the foreseeable future it cannot be offered to patients

Conclusion

Ovarian tissue cryopreservation is a feasible option to preserve ovarian function and future fertility in children, premenarchal girls as well as young women before they undertake gonadotoxic chemotherapy and/or radiotherapy. To date more than 30 pregnancies have been reported after autotransplantation of ovarian cortical tissue slices in young women. However, there are still several challenges to meet and further intensive research is needed to assess the clinical effectiveness of ovarian cryopreservation, to optimise the technique and increase its safety.

muhammad.fatum@obs-gyn.ox.ac.uk

Key Practice Points

1. Ovarian tissue cryopreservation can offer an alternative to ovarian stimulation and oocyte harvesting when cancer treatment cannot be delayed.
2. Recent evidence suggests that vitrification may be superior to slow freezing of ovarian tissue.
3. Thawed ovarian tissue should be tested for micrometastasis prior to transplantation.
4. Transplantation of ovarian tissue may be orthotopic or heterotopic.

References

1. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, Beck LN, Brennan LV, Oktay K. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol. 2006;24(18):2917–31.
2. Kim SS, Donnez J, Barri P, Pellicer A, Patrizio P, Rosenwaks Z, Nagy P, Falcone T, Andersen C, Hovatta O, Wallace H, Meirow D, Gook D, Kim SH, Tzeng CR, Suzuki S, Ishizuka B, Dolmans MM. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet. 2012;29(6):465–8.
3. Meirow D, Hardan I, Dor J, Fridman E, Elizur S, Ra'anani H, Slyusarevsky E, Amariglio N, Schiff E, Rechavi G, Nagler A, Ben Yehuda D. Searching for evidence of disease and malignant cell contamination in ovarian tissue stored from hematologic cancer patients. Hum Reprod. 2008;23(5):1007–13.
4. Nisolle M, Casanas-Roux F, Qu J, Motta P, Donnez J. Histologic and ultrastructural evaluation of fresh and frozen-thawed human ovarian xenografts in nude mice. Fertil Steril. 2000;74:122–9.
5. Dolmans MM, Marinescu C, Saussoy P, Van Langendonckt A, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukaemia is potentially unsafe. Blood. 2010;116(16):2906–14.
6. Sukur YE, Ozmen B, Senmezer M. Cancer and ovarian tissue cryopreservation. Turk J Med Sci. 2010; 40(2):159–68.
7. Herraiz S, Novella-Maestre E, Rodríguez B, Díaz C, Sánchez-Serrano M, Mirabet V, Pellicer A. Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices. Fertil Steril. 2014;101(3):775–84.
8. von Wolff M. Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy – a technique in its infancy but already successful in fertility preservation. Eur J Cancer. 2009;45:1547–53.
9. Tulandi T, Gosden R. Preservation of fertility. London: Taylor & Francis; 2004.
10. Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. Reprod Biomed Online. 2011;22:162–71.
11. Hovatta O. Methods for cryopreservation of human ovarian tissue. Reprod Biomed Online. 2005;10(6):729–34.
12. Amorim CA, Jacobs S, Devireddy RV, Van Langendonckt A, Vanacker J, Jaeger J, Luyckx V, Donnez J, Dolmans MM. Successful vitrification and autografting of baboon (Papio anubis) ovarian tissue. Hum Reprod. 2013;28(8): 2146–56.
13. Amorim CA, Curaba M, Van Langendonckt A, Dolmans MM, Donnez J. Vitrification as an alternative means of cryopreserving ovarian tissue. Reprod Biomed Online. 2011;23(2):160–86.
14. Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. Molec Hum Reprod. 2012;18(2):59–67.
15. Revel A, Laufer N, Ben Meir A, Lebovich M, Mitrani E. Micro-organ ovarian transplantation enables pregnancy: a case report. Hum Reprod. 2011;26(5): 1097–103.
16. Candy CJ, Wood MJ, Whittingham DG. Effect of cryoprotectants on the survival of follicles in frozen mouse ovaries. J Reprod Fertil. 1997;110:11–9.
17. Hovatta O, Silye R, Krausz T, Abir R, Margara R, Trew G, Lass A, Winston RM. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. Hum Reprod. 1996;11(6):1268–72.
18. Azem F, Hasson J, Ben-Yosef D, Kossoy N, Cohen T, Almog B, Amit A, Lessing JB, Lifschitz-Mercer B. Histologic evaluation of fresh human ovarian tissue before cryopreservation. Int J Gynecol Pathol. 2010;29:19–23.
19. Merdassi G, Mazoyer C, Guerin JF, Saad A, Salle B, Lornage J. Examination of viability and quality of ovarian tissue after cryopreservation using simple laboratory methods in ewe. Reprod Biol Endocrinol. 2011;9:1–7.
20. Zhang JM, Sheng Y, Cao YZ, Wang HY, Chen ZJ. Effects of cooling rates and ice-seeding temperatures on the cryopreservation of whole ovaries. J Assist Reprod Genet. 2011;28:627–33.
21. Newton H, Fisher J, Arnold JR, Pegg DE, Faddy MJ, Gosden RG. Permeation of human ovarian tissue with cryoprotective agents in preparation for cryopreservation. Hum Reprod. 1998;13(2):376–80.
22. Shaw JM, Oranratnachai A, Trounson AO. Fundamental cryobiology of mammalian oocytes and ovarian tissue. Theriogenology. 2000;53:59–72.
23. Tedder RS, Zuckerman MA, Goldstone AH, Hawkins AE, Fielding A, Briggs EM, Irwin D, Blair S, Gorman AM, Patterson KG. Hepatitis B transmission from contaminated cryopreservation tank. Lancet. 1995;346:137–40.

24. Sonmezer M, Oktay K. Fertility preservation in female patients. Hum Reprod Update. 2004;10(3):251–66.
25. Revel A, Revel-Vilk S, Aizenman E, Porat-Katz A, Safran A, Ben-Meir A, Weintraub M, Shapira M, Achache H, Laufer N. At what age can human oocytes be obtained? Fertil Steril. 2009;92(2):458–63.
26. Meirow D, Baum M, Yaron R, Levron J, Hardan I, Schiff E, Nagler A, Yehuda DB, Raanani H, Hourvitz A, Dor J. Ovarian tissue cryopreservation in hematological malignancy: ten years experience. Leuk Lymphoma. 2007;48(8):1569–76.
27. Revel A, Revel-Vilk S. Fertility preservation in young cancer patients. J Hum Reprod Sci. 2010;3(1):2–7.
28. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue banking activities. J Assist Reprod Genet. 2013;30(3):305–14.
29. Donnez J, Jadoul P, Squifflet J, Van Langendonckt A, Donnez O, Van Eyck AS, Marinescu C, Dolmans MM. Ovarian tissue cryopreservation and transplantation in cancer patients. Best Pract Res Clin Obstet Gynaecol. 2010;24:87–100.
30. Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, Opsahl M, Rosenwaks Z. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;363:837–40.
31. Demeestere I, Simon P, Emiliani S, Delbaere A, Englert Y. Orthotopic and heterotopic ovarian tissue transplantation. Hum Reprod Update. 2009;15(6):649–65.
32. Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, van Langendonckt A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet. 2004;364:1405–10.
33. Donnez J, Squifflet J, Van Eyck AS, Demylle D, Jadoul P, Van Langendonckt A, Dolmans MM. Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience. Reprod Biomed Online. 2008;16:694–704.
34. Schmidt KL, Andersen CY, Loft A, Byskov AG, Ernst E, Andersen AN. Follow up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. Hum Reprod. 2005;20:3539–46.
35. Tryde Schmidt KL, Yding Andersen C, Starup J, Loft A, Byskov AG, Nyboe Andersen A. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer—follicular growth, steroid production and oocyte retrieval. Reprod Biomed Online. 2004;8:448–53.
36. Oktay K, Newton H, Gosden RG. Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice. Fertil Steril. 2000; 73:599–603.
37. Radford JA, Lieberman BA, Brison DR, Smith AR, Critchlow JD, Russell SA, Watson AJ, Clayton JA, Harris M, Gosden RG, et al. Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet. 2001;357:1172–5.
38. Rosendahl M, Loft A, Byskov AG, Ziebe S, Schmidt KT, Andersen AN, Ottosen C, Andersen CY. Biochemical pregnancy after fertilization of an oocyte aspired from a heterotopic autotransplant of cryopreserved ovarian tissue: case report. Hum Reprod. 2006;21:2006–9.
39. Newton H, Aubard Y, Rutherford A, Sharma V, Gosden R. Low temperature storage and grafting of human ovarian tissue. Hum Reprod. 1996;11: 1487–91.
40. Aubard Y, Piver P, Cogni Y, Fermeaux V, Poulin N, Driancourt MA. Orthotopic and heterotopic autografts of frozen–thawed ovarian cortex in sheep. Hum Reprod. 1999;14:2149–54.
41. Donnez J, Martinez-Madrid B, Jadoul P, Van Langendonckt A, Demylle D, Dolmans MM. Ovarian tissue cryopreservation and transplantation: a review. Hum Reprod Update. 2006;12:519–35.
42. Nottola SA, Camboni A, Van Langendonckt A, Demylle D, Macchiarelli G, Dolmans MM, Martinez-Madrid B, Correr S, Donnez J. Cryopreservation and xenotransplantation of human ovarian tissue: an ultrastructural study. Fertil Steril. 2008;90:23–32.
43. Van Eyck AS, Bouzin C, Feron O, Romeu L, Van Langendonckt A, Donnez J, Dolmans MM. Both host and graft vessels contribute to revascularization of xenografted human ovarian tissue in a murine model. Fertil Steril. 2010;93:1676–85.
44. Dolmans MM, Martinez-Madrid B, Gadisseux E, Guiot Y, Yuan WY, Torre A, Camboni A, Van Langendonckt A, Donnez J. Short term transplantation of isolated human ovarian follicles and cortical tissue into nude mice. Reproduction. 2007;134:253–62.
45. David A, Dolmans MM, Van Langendonckt A, Donnez J, Amorim CA. Immunohistochemical localization of growth factors after cryopreservation and 3 weeks' xenotransplantation of human ovarian tissue. Fertil Steril. 2011;95:1241–6.
46. des Rieux A, Ucakar B, Mupendwa BP, Colau D, Feron O, Carmeliet P, Préat V. 3D systems delivering VEGF to promote angiogenesis for tissue engineering. J Control Release. 2011;30:272–8.
47. Dath C, Dethy A, Van Langendonckt A, Van Eyck AS, Amorim CA, Luyckx V, Donnez J, Dolmans MM. Endothelial cells are essential for ovarian stromal tissue restructuring after xenotransplantation of isolated ovarian stromal cells. Hum Reprod. 2011;26: 1431–9.
48. Roness H, Gavish Z, Cohen Y, Meirow D. Ovarian follicle burnout: a universal phenomenon? Cell Cycle. 2013;12(20):3245–6.
49. Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO. Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit cancer to graft recipients. Hum Reprod. 1996;11:1668–73.
50. Xu M, Kreeger PK, Shea LD, Woodruff TK. Tissue-engineered follicles produce live, fertile offspring. Tissue Eng. 2006;12:2739–46.
51. Xu M, Barrett SL, West-Farrell E, Kondapalli LA, Kiesewetter SE, Shea LD, Woodruff TK. *In-vitro* grown human ovarian follicles from cancer patients support oocyte growth. Hum Reprod. 2009;24(10): 2531–40.

muhammad.fatum@obs-gyn.ox.ac.uk

the ovarian surface in infertile patients with polycystic ovarian disease. Fertil Steril. 1993;60:88–94.
25. Amer SA, Li TC, Cooke ID. Laparoscopic ovarian diathermy in women with polycystic ovarian syndrome: a retrospective study on the influence of the amount of energy used on the outcome. Hum Reprod. 2002;17:1046–51.
26. Amer S, Li TC, Cooke ID. A prospective dose finding study of the amount of energy required for laparoscopic ovarian diathermy in women with polycystic ovarian syndrome. Hum Reprod. 2003;18:1693–8.
27. Felemban A, Tan SL, Tulandi T. Laparoscopic treatment of polycystic ovaries with insulated needle cautery: a reappraisal. Fertil Steril. 2000;73(2):266–9.
28. Greenblatt E, Casper RF. Endocrine changes after laparoscopic ovarian cautery in polycystic ovarian syndrome. Am J Obstet Gynaecol. 1987;156:279–85.
29. Lemieux S, Lewis GF, Ben-Chetrit A, Steiner G, Greenblatt EM. Correction of hyperandrogenemia by laparoscopic ovarian cautery in women with polycystic ovarian syndrome is not accompanied by improved insulin sensitivity or lipid-lipoprotein levels. J Clin Endocrinol Metab. 1999;84:4278–82.
30. Pelosi MA, Pelosi MA. Laparoscopic electrosurgical furrowing technique for the treatment of polycystic ovaries. J Am Assoc Gynaecol Laparosc. 1996;4:57–62.
31. Merchant RN. Treatment of polycystic ovary disease with laparoscopic low-watt bipolar electrocoagulation of the ovaries. J Am Assoc Gynaecol Laparosc. 1996; 3:503–8.
32. Gurgan T, Kisnisci H, Yarali H, Develioglu O, Zeyneloglu H, Aksu T. Evaluation of adhesion formation after laparoscopic treatment of polycystic ovarian disease. Fertil Steril. 1991;56:1176–8.
33. Keckstein G, Rossmanith W, Spatzier K, Schneider V, Borchers K, Steiner R. The effect of laparoscopic treatment of polycystic ovarian disease by CO_2- laser or Nd:YAG laser. Surg Endosc. 1990;4:103–7.
34. Keckstein J. Laparoscopic treatment of polycystic ovarian syndrome. Baillieres Clin Obstet Gynaecol. 1989;3:563–81.
35. Kojima E, Yanagibori A, Otaka K, Hirakawa S. Ovarian wedge resection with contact Nd:YAG laser irradiation used laparoscopically. J Reprod Med. 1989;34:444–6.
36. Li TC, Saravelos H, Chow MS, Chisabingo R, Cooke ID. Factors affecting the outcome of laparoscopic ovarian drilling for polycystic ovarian syndrome in women with anovulatory infertility. Br J Obstet Gynaecol. 1998;105:338–44.
37. Naether OGJ, Baukloh V, Fischer R, Kowalczyk T. Long-term follow-up in 206 infertility patients with polycystic ovarian syndrome after laparoscopic electrocautery of the ovarian surface. Hum Reprod. 1994;9:2342–9.
38. Mio Y, Toda T, Tanikawa M, Terado H, Harada T, Terakawa N. Transvaginal ultrasound-guided follicular aspiration in the management of anovulatory infertility associated with polycystic ovaries. Fertil Steril. 1991;56:1060–5.
39. Syritsa A. Transvaginal ultrasound-guided electrocautery of the ovaries in infertile patients with polycystic ovarian disease. Int J Gynaecol Obstet. 1998;63: 293–4.
40. Zhu W, Li X, Chen X, Lin Z, Zhang L. Ovarian interstitial YAG-laser: an effective new method to manage anovulation in women with polycystic ovary syndrome. Am J Obstet Gynecol. 2006;195:458–63.
41. Fernandez H, Watrelot A, Alby JD, et al. Fertility after ovarian drilling by transvaginal fertiloscopy for treatment of polycystic ovary syndrome. J Am Assoc Gynecol Laparosc. 2004;11:374–8.
42. Ramzy AM, Al-Inany H, Aboulfoutouh I, Sataar M, Idrees OA, Shehata MH. Ultrasonographic guided ovarian stroma hydrocoagulation for ovarian stimulation in polycystic ovary syndrome. Acta Obstet Gynecol Scand. 2001;80:1046–50.
43. Chiesa-Montadou S, Rongieres C, Garbin O, Nisand I. About two complications of ovarian drilling by fertiloscopy. Gynecol Obstet Fertil. 2003;31:844–6.
44. Kovacs G, Buckler H, Bangah M, et al. Treatment of anovulation due to polycystic ovarian syndrome by laparoscopic ovarian electrocautery. Br J Obstet Gynaecol. 1991;98:30–5.
45. Liguori G, Tolino A, Moccia G, Scognamiglio G, Nappi C. Laparoscopic ovarian treatment in infertile patients with polycystic ovarian syndrome (PCOS): endocrine changes and clinical outcome. Gynaecol Endocrin. 1996;10:257–64.
46. Aakvaag A, Gjonnaess H. Hormonal response to electrocautery of the ovary in patients with polycystic ovarian disease. Br J Obstet Gynaecol. 1985;92:1258–64.
47. Gjonnaess H, Norman N. Endocrine effects of the ovarian electrocautery in patients with polycystic ovarian disease. Br J Obstet Gynaecol. 1987;94:779–83.
48. Abdel-Gadir A, Mowafi RS, Alnaser HMI, Alrashid AH, Alonezi OM, Shaw RW. Ovarian electrocautery versus human gonadotrophins and pure follicle stimulating hormone therapy in the treatment of patients with polycystic ovarian disease. Clin Endocrinol (Oxf). 1990;33:585–92.
49. Amer S, Laird S, Ledger WL, Li TC. Effect of laparoscopic ovarian diathermy on circulating inhibin B in women with anovulatory polycystic ovarian syndrome. Hum Reprod. 2007;22:389–94.
50. Gjonnaess H. Late endocrine effects of ovarian electrocautery in of women with polycystic ovary syndrome. Fertil Steril. 1998;69:697–701.
51. Elting MW, Korsen TJM, Rekers-Mombarg LTM, Schoemaker J. Women with polycystic ovary syndrome gain regular menstrual cycles when ageing. Hum Reprod. 2000;15:24–8.
52. Amer S, Li TC, Banu Z, Cooke ID. Long term follow up of patients with polycystic ovarian syndrome after laparoscopic ovarian drilling: endocrine and ultrasonographic outcomes. Hum Reprod. 2002;17:2851–7.
53. Amer S, Li TC, Gopalan V, Ledger WL, Cooke ID. Long term follow up of patients with polycystic ovarian syndrome after laparoscopic ovarian drilling: clinical outcome. Hum Reprod. 2002;17:2035–42.

54. Fukaya T, Murakami T, Tamura M, Watanabe T, Terada Y, Yajima A. Laser vaporization of the ovarian surface in polycystic ovary disease results in reduced ovarian hyperstimulation and improved pregnancy rates. Am J Obstet Gynecol. 1995;173:119–25.
55. Colacurci N, Zullo F, De Franciscis P, Mollo A, De Placido G. In vitro fertilization following laparoscopic ovarian diathermy in patients with polycystic ovarian syndrome. Acta Obstet Gynecol Scand. 1997;76:555–8.
56. Tozer AJ, Al-Shawaf T, Zosmer A, Hussain S, Wilson C, Lower AM, Grudzinskas JG. laparoscopic ovarian diathermy affect the outcome of IVF embryo transfer in women with polycystic ovarian syndrome? A retrospective comparative study. Hum Reprod. 2001;16:91–5.
57. Rimington MR, Walker SM, Shaw RW. The use of laparoscopic ovarian electrocautery in preventing cancellation of in vitro fertilization treatment cycles due to risk of ovarian hyperstimulation syndrome in women with polycystic ovaries. Hum Reprod. 1997; 12:1443–7.
58. Amin AF, Abdel-Aal DE, Darwish AM, Mbeki AR. Evaluation of the impact of laparoscopic ovarian drilling on Doppler indices of ovarian stoma blood flow, serum vascular endothelial growth factor, and insulin-like growth factor-1 in women with polycystic ovary syndrome. Fertil Steril. 2003;79:938–42.
59. Parsanezhad ME, Bagheri MH, Alborzi S, Schmidt EH. Ovarian stromal blood flow changes after laparoscopic ovarian cauterization in women with polycystic ovary syndrome. Hum Reprod. 2003;18:1432–7.
60. El Behery MM, Diab AE, Mowafy H, Ebrahiem MA, Shehata AE. Effect of laparoscopic ovarian drilling on vascular endothelial growth factor and ovarian stromal blood flow using 3-dimensional power Doppler. Int J Gynaecol Obstet. 2011;112:119–21.
61. Rossmanith WG, Keckstein J, Spatzier K, Lauritzen C. The impact of ovarian laser surgery on the gonadotrophin secretion in women with polycystic ovarian disease. Clin Endocrinol (Oxf). 1991;34:223–30.
62. Amer S, Li TC, Ledger WL. Ovulation induction using laparoscopic ovarian drilling in women with polycystic ovarian syndrome: predictors of success. Hum Reprod. 2004;19:1719–24.
63. Amer S, Li TC, Ledger WL. The value of measuring anti-Müllerian hormone in women with anovulatory polycystic ovary syndrome undergoing laparoscopic ovarian diathermy. Hum Reprod. 2009;24:2760–6.
64. Greenblatt EM, Casper RF. Adhesion formation after laparoscopic ovarian cautery for polycystic ovarian syndrome: lack of correlation with pregnancy rate. Fertil Steril. 1993;60:766–70.
65. Naether OGJ. Significant reduction of adnexal adhesions following laparoscopic electrocautery of the ovarian surface (LEOS) by lavage and artificial ascites. Gynaecol Endosc. 1995;4:17–9.
66. Saravelos H, Li TC. Post-operative adhesions after laparoscopic electrosurgical treatment for polycystic ovarian syndrome with the application of Interceed to one ovary: a prospective randomized controlled study. Hum Reprod. 1996;11:992–7.
67. Amer S, Li TC, Cooke ID. Repeated laparoscopic ovarian diathermy is effective in women with anovulatory infertility due to polycystic ovarian syndrome. Fertil Steril. 2003;79:1211–5.

European Journal of Obstetrics & Gynecology and Reproductive Biology 230 (2018) 10–14

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Full length article

Ovarian response and follow-up outcomes in women diagnosed with cancer having fertility preservation: Comparison of random start and early follicular phase stimulation - cohort study

Charles Muteshi[a,b,c,*], Tim Child[a,b], Eric Ohuma[d,e], Muhammad Fatum[a,b]

[a] Oxford Fertility, Institute of Reproductive Sciences, Oxford OX4 2HW, UK
[b] Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford OX3 9DU, UK
[c] Endometriosis CaRe Centre, Oxford OX3 9DU, UK
[d] Centre for Statistics in Medicine, Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology & Musculoskeletal Sciences, University of Oxford, Oxford, UK
[e] Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK

ARTICLE INFO

Article history:
Received 17 February 2018
Received in revised form 2 July 2018
Accepted 9 September 2018

Keywords:
Fertility preservation
Cancer
Random start stimulation

ABSTRACT

Objectives: To determine response to controlled ovarian stimulation in a random start cycle and utilisation of cryopreserved oocytes and embryos in cancer patients.
Study Design: A retrospective cohort study was carried out in an assisted reproductive treatment centre. Participants included 137 cancer patients who underwent controlled ovarian stimulation for fertility preservation between 1 Feb 2003 and 30 June 2016. The primary outcome variable was number of oocytes retrieved. Multivariable logistic regression analysis was performed, and differences compared using Chi squared test and student *t*-test as appropriate. $P < 0.05$ was considered statistically significant.
Results: Using the antagonist protocol, there was no difference in number of oocytes retrieved between the early follicular phase or at random start stimulation; 11.9 (95% CI 10.3–13.5) and 12.9 (95% CI 9.6–16.2), $P = 0.602$, respectively. Similarly, the number of embryos frozen was comparable between those starting stimulation in early follicular and random phase, 6.7 (95% CI 5.7–7.7) and 5.1 (95% CI 3.6–6.5), P= 0.1508 respectively. Among patients undergoing fertility preservation, those who returned to attempt a pregnancy had an ongoing pregnancy rate of 24.3%. Overall, 65% of oocytes and embryos were still in storage, however, 16 (11.7%) had elected to have their oocytes or embryos disposed of.
Conclusion(s): For women faced with potential gonadotoxic treatment and requiring urgent fertility preservation, ovarian stimulation with the antagonist protocol can be started at random without compromising ovarian response. Pregnancy rates following utilisation of frozen-thawed oocytes and embryos are promising, however, more research is needed to understand reasons underlying disposition of oocytes and embryos especially when survival following cancer treatment has improved significantly.

Introduction

Over the past few decades developments in cancer treatment protocols have resulted in improved long-term survival of cancer patients, however, due to their variable gonadotoxicity these treatments may leave some survivors with severely compromised ovarian function [1]. Several studies show that women of reproductive age would like the opportunity to discuss fertility implications of their cancer diagnosis and treatment [2]. Although an increasing number of oncologists discuss fertility implications with patients, the overarching urgency to treat cancer implies that not all patients have the opportunity to explore fertility preservation [3]. Uniquely for women with hormone receptor positive breast cancer there is the additional challenge of having to take the anti-oestrogen tamoxifen for several years during which pregnancy is contraindicated. Since natural fertility declines with age, any compromise in ovarian function may only become apparent when trying for a pregnancy years later after completing tamoxifen therapy [4,5].

Opportunities for fertility preservation prior to gonadotoxic treatment include: vitrification of oocytes and/ or embryos, ovarian tissue cryopreservation and concomitant administration

* Corresponding author at: University of Oxford, Nuffield Department of Women's and Reproductive Health, Level 3 Women's Centre, Headington, Oxford OX3 9DU, UK.
E-mail address: charles.muteshi@obs-gyn.ox.ac.uk (C. Muteshi).

https://doi.org/10.1016/j.ejogrb.2018.09.007
0301-2115/

of gonadotropin releasing hormone agonist (GnRH-a) with chemotherapy [6]. Offering a combination of these methods is recommended as a means of maximizing patients' chances of future fertility, rather than choosing one only. Cryopreservation of oocytes or embryos requires a woman undergoes a cycle of controlled ovarian stimulation using either of various protocols [7]. The long agonist protocol is less favoured now as it requires a longer duration of treatment due to initial pituitary down-regulation and has a higher risk of ovarian hyperstimulation syndrome (OHSS) [8]. Conversely, the antagonist protocol is devoid of initial pituitary down regulation and has a lower risk of OHSS, rendering it the preferred protocol for fertility preservation programmes [9].

Initiation of ovarian stimulation usually starts in the early follicular phase of the menstrual cycle based on the long-held view that recruitment of selectable follicles happens in this phase. However, observations made in animals as well as humans challenge this theory by demonstrating a continuous growth of antral follicles throughout the oestrous/menstrual cycles [10–12]. This was the basis of reports indicating that ovarian stimulation started in the luteal had a comparable number of mature oocytes and embryo development to early follicular start [13]. This enabled development of random start ovarian stimulation protocols for cancer patients who require urgent treatment [14]. The described random-start protocols are complex therefore there is a need for a more simplified approach.

This study aimed to establish whether response to controlled ovarian stimulation was comparable between random start, early follicular and luteal phase antagonist cycles in women needing urgent fertility preservation using a simplified protocol. Secondary outcomes included adverse events and the outcome of cryopreserved oocytes and/ or embryos.

Materials and methods

Study population

We undertook a comprehensive analysis of clinical data that is prospectively entered and stored in a database (Paradox, Borland, Scott Valley, CA, USA) at Oxford Fertility. All patients recently diagnosed with cancer and referred for fertility preservation between Feb 1, 2003 and June 30, 2016 were included in this retrospective cohort study. Approval was sought from the University of Oxford Central University Research Ethics Committee.

Treatment protocol

After a fertility specialist medical consultation, patients attend nurse treatment planning appointment as soon as possible. Treatment initiation starts immediately after this planning on the agreement of the oncologist.

The conventional antagonist protocol at Oxford Fertility during the period of the study was as follows. On day two to five of the menstrual cycle, daily ovarian stimulation with gonadotrophin (recombinant gonadotrophin alpha - Gonal F®, Serono Pharmaceuticals Ltd., Feltham, UK; Puregon®, Organon Laboratories Ltd., Hoddesdon, UK, or Menopur®, Ferring Pharmaceuticals Ltd., West Drayton, UK) was commenced. After five days of stimulation, cetrorilex acetate 250-mcg subcutaneous injection (Cetrotide®, Serono Pharmaceuticals Ltd, Feltham, UK) was introduced and continued until human chorionic gonadotrophin or buserelin for final oocyte maturation was administered. Monitoring follicle development with transvaginal ultrasound scanning and serum oestradiol started after the sixth day following the initiation of stimulation. When at least three follicles measuring ≥18 mm were seen on ultrasound scan, human chorionic gonadotropin (hCG) 6500 IU (Ovitrelle®; Serono Pharmaceuticals Ltd., Feltham, UK) or buserelin 0.5 mg was administered subcutaneously for final oocyte maturation 37 h prior to oocyte-cumulus complex retrieval. The practice was to aspirate from all follicles measuring ≥12 mm. Oocytes were then stripped clear of the cumulus cells within two hours of oocyte retrieval for women choosing oocytes vitrification, whereas those opting to freeze embryos micro-injection of oocytes with sperm was done after four hours. Fertilisation check followed 16 h from oocyte microinjection.

The random start protocol was used if stimulation started at any point after menstrual day five. It involved administration of cetrorilex acetate 250 mcg (Cetrotide®, Serono Pharmaceuticals Ltd, Feltham, UK) for three days followed by ovarian stimulation with gonadotrophin from the fourth day. The cetrorilex acetate was continued together with the gonadotrophin stimulation until ovarian response was deemed appropriate for HCG or buserelin administration as described above. Follicle growth monitoring was as for the conventional antagonist protocol starting after six days of stimulation.

Gonadotrophin dose determination

The starting dose of gonadotrophins was based on female age, body mass index (BMI), antral follicle count or anti-Mullerian hormone (AMH) concentration. Patients were stimulated on a dose ranging from 150 IU to 375 IU of Gonal-F® or Menopur®.

Outcome measures

The primary outcome measure was the number of oocytes retrieved per started cycle. We also analysed the duration from referral to oocyte retrieval; starting and total dose of gonadotrophin; duration of ovarian stimulation; number of follicles measuring ≥15 mm and peak oestradiol concentration at the time of HCG or buserelin trigger; number of fertilised oocytes and number of oocytes or embryos put in storage. For the oocytes and embryos cryopreserved, we computed those remaining in storage at the time of our analysis and determined fate of the rest. We also assessed immediate complications following oocyte retrieval.

Statistical analysis

For categorical variables, we calculated percentages and compared differences using the Chi-squared or Fisher's exact test as appropriate. For the continuous variables, we calculated their mean and compared differences using the Students *t*-test. All statistical analysis was performed in statistical software STATA® version 12 (College Station, TX, USA). *P* value <0.05 was considered significant.

Results

Baseline characteristics

We reviewed 137 records of patients who presented for fertility preservation prior to starting gonadotoxic treatment between February 1, 2003 and June 30, 2016. Nine of these had the long agonist protocol and were excluded from analysis of the main outcome variables. The remaining 127 women were on the antagonist protocol (103 in the conventional start group and 24 in the random start group). The baseline characteristics of the women on the antagonist protocol are shown in Table 1. There were no statistically significant differences in baseline characteristics of women in the two treatment groups. The mean age for women in the groups was similar, with age ranging between 18.5–

Table 1
Baseline characteristics.

Variable	Conventional protocol N = 103 (95% CI)	Random start protocol N = 24 (95% CI)
Age (years)	32.6 (31.4–33.6)	30.5 (28.3–32.8)
FSH (IU/L)	6.6 (4.9–8.1)	5.9 (1.2–10.5)
AFC	16.4 (14.5–18.3)	19.3 (14.9–23.6)
AMH (pmol/L)	18.6 (12.0–25.1)	13.0 (4.6–21.4)
BMI (kg/m^2)	23.5 (22.7–24.1)	23.6 (21.1–26.1)
Number of children	0.17	0.16

Legend: AFC (antral follicle count); AMH (anti-Mullerian hormone); BMI (body mass index); FSH (follicle stimulating hormone).

42.5 years. Similarly, markers of ovarian reserve (FSH, antral follicle count and AMH) were comparable between the groups. Fig. 1 shows the proportion of various cancer diagnoses for the complete cohort of 137 women. Breast cancer was the commonest underlying referral for fertility preservation.

Main outcome variables

To determine any differences between conventional and random start cycles using the antagonist protocol, we excluded the long agonist cycles from the main analysis. A total of 127 antagonist cycles were included in the outcomes as shown in Table 2.

There was no statistically significant difference between the conventional and random start cycles in the number of oocytes retrieved 11.9 (95% CI 10.3–13.5) and 12.9 (95% CI 9.6–16.2); P = 0.602. Additionally, there was no statistically significant difference for average numbers of embryos frozen per cycle between the two groups at 6.7 (95% CI 5.7–7.7) and 5.1 (95% 3.6–6.5); P = 0.151, respectively. It took an average of one month from the date of oncologist referral to the oocyte retrieval in all the groups. This is likely due to an over-representation of breast cancer patients who had surgery prior to starting ovarian stimulation. There was no difference between the two groups in other cycle characteristics during controlled ovarian stimulation as shown in Table 2. For women who had controlled ovarian stimulation on the antagonist protocol, 91.3% in the conventional and 83.3% in the random start groups opted to cryopreserve embryos while the rest vitrified oocytes (data not shown).

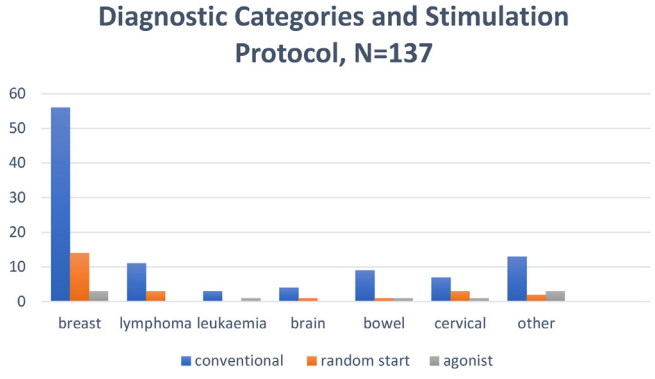


Fig. 1. Disease categories and stimulation protocol.

We reviewed the adverse events that occurred during controlled ovarian stimulation, oocyte retrieval and up to six weeks following completion of the treatment cycle. These included cycle cancellation, OHSS, complications of oocyte retrieval (such as bleeding, infection or perforation of visceral structures) or no oocytes and/or embryos available to freeze. As these events were rare, we analysed them according to major cancer diagnostic categories as the urgency of treatment since diagnosis may be different. There were four cycles without oocytes or embryos available for cryopreservation, including one cycle cancelled prior to oocyte retrieval. Only two cases of moderate OHSS were recorded while one woman was admitted with leg pain, however, a diagnosis of deep venous thrombosis was ruled out, Table 3.

We also looked to determine the fate of oocytes and embryos in cryo-storage for all the treatment cycles as this was independent of ovarian stimulation protocol. Of the 137 women who underwent fertility preservation, 89 (65%) patients had oocytes or embryos still in storage at the time of review. Fifteen patients had returned to the clinic for oocytes or embryos thaw-transfer in 37 treatment cycles. This resulted in 29.7% (11 of 37) implantation rate and 24.3% (9 of 37) ongoing pregnancy rate. Due to a variety of unknown reasons sixteen women had given consent to discard embryos from storage while three had their oocytes or embryos transferred elsewhere. Sadly, eleven women were deceased, representing eight percent of all patients (data not shown) (Fig. 2).

Table 3
Adverse treatment outcomes.

Adverse outcome	Breast N = 72	Lymphoma N = 15	Leukaemia N = 4	Other cancers N = 36
Cycle cancellation				
No	71	15	4	36
Yes	1	0	0	0
OHSS				
None	71	14	4	34
Mild	0	1	0	1
Moderate	1	0	0	1
Complications (bleeding/infection/other)				
No	71	15	4	36
Yes	1[a]	0	0	0
Nothing to freeze				
No	69	15	4	35
Yes	2	0	0	1

[a] Admitted with leg pain. OHSS (ovarian hyperstimulation syndrome).

Table 2
Outcome variables.

Outcome variable	Conventional N = 103 (95% CI)	Random start N = 24 (95% CI)	P value[a]
Oncology referral -OCR (days)	33.5 (27.9–38.9)	33.9 (24.8–42.8)	N/S
Duration of stimulation (days)	11.5 (11.2–12.0)	12.2 (10.7–13.7)	N/S
Total dose of FSH (IU)	2543.4 (2328.3–2758.5)	2811.9 (2090.8–3533.1)	N/S
Number of follicles 15 mm or greater at HCG trigger	9.06 (8.04–10.1)	10.6 (8.6–12.6)	N/S
Oestradiol at HCG trigger (pmol/L)	5426.3 (4682.9–6169.7)	4423.1 (2866.9–5979.3)	N/S
Number of oocytes	11.9 (10.3–13.5)	12.9 (9.6–16.2)	N/S
Number fertilised	7.2 (6.1–8.3)	5.9 (4.3–7.6)	N/S
Number frozen	6.7 (5.7–7.7)	5.1 (3.6–6.5)	N/S

Legend: FSH (follicle stimulating hormone); HCG (human chorionic gonadotropin); OCR (oocyte cumulus retrieval).
[a] Denotes reason for admission to hospital, this is not one of the listed complications.

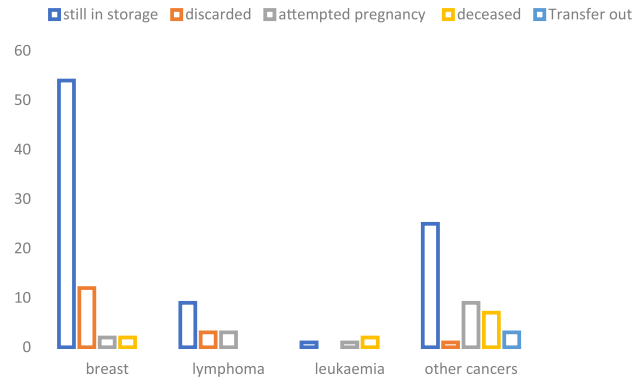


Fig. 2. Fate of cryopreserved oocytes/embryos.

Comment

Controlled ovarian stimulation for oocyte or embryo cryopreservation is an established technique for fertility preservation in cancer patients [3]. Conventionally, ovarian stimulation is started in the early follicular phase of the menstrual cycle. Women diagnosed with cancer and faced with potential gonadotoxic treatment present for fertility preservation at various times in their menstrual cycle. Waiting for the follicular phase to start ovarian stimulation may pose a significant delay in commencing cancer treatment. Our study demonstrates that ovarian stimulation using the antagonist protocol in a simplified random start protocol is comparable to the early follicular phase start.

These findings are similar to those of previous case control studies in which women underwent ovarian stimulation on the antagonist protocol starting either in the early follicular phase, late follicular phase or the luteal phase of the menstrual cycle for fertility preservation [13,15]. While these authors found that stimulation started randomly or in the luteal phase took longer than when started in the early follicular phase, our study showed no statistically significant difference in the duration of stimulation and total gonadotrophin dosage used.

Our study confirms the observation that follicular recruitment can be initiated at any point in the menstrual cycle and further debunks the long-held view that this is limited to the early follicular phase [11]. Baerwald et al. demonstrated that follicular recruitment could be initiated at any point in the menstrual cycle when they observed multiple waves of follicular activity in both the luteal and follicular phase of women's menstrual cycles [12]. For women diagnosed with cancer there is often a necessity to start and complete controlled ovarian stimulation within tight time frames and waiting for the early follicular phase may lead to unwarranted delay or foregoing of the only opportunity available for fertility preservation.

Previous studies reported on luteal phase stimulation using the antagonist protocol with results comparable to stimulation started in the early follicular phase [16–18]. However, women presenting after the early follicular phase had to wait for the luteal phase to start ovarian stimulation. This dilemma was overcome as described by a case series of women with breast cancer where emergency stimulation starting on days 11, 14 and 17 resulted in an acceptable number of oocytes and embryos available for cryopreservation [13]. Subsequently, case controlled studies confirmed that random start stimulation protocols have comparable outcomes to those of the conventional follicular or luteal phase stimulation [14,19]. There are complex variations in the methodologies for the luteal and late follicular start cycles as reported in these studies as well as the earlier reported luteal phase start cycle. In the Cakmak et al. study for instance late follicular phase stimulation was started if no leading follicle of greater than 12 mm was present, and the antagonist introduced when the follicle cohort reached 12 mm. If there was a larger follicle, ovulation was induced with HCG or GnRH agonist followed by gonadotrophin stimulation two to three days later [14]. Whereas Kim et al. started ovarian stimulation on the day of presentation regardless of the cycle day [19]. In another study the authors reported that women with a formed corpus luteum were given the GnRH antagonist to suppress LH activity and induce luteolysis followed by ovarian stimulation simultaneously or after three days [15]. These differences between the random and luteal start protocols are complex, prone to cause confusion among clinicians and burdensome to patients.

Controlled ovarian stimulation and oocyte retrieval are not without risk, and cancer patients are in a particularly precarious position due to time constraints [20,21]. The commonest and serious complication of these procedures is OHSS which occurs in around 0.6–5% of cycles and may lead to prolonged hospitalisation and rarely mortality [22]. Using the antagonist protocol together with GnRH agonist trigger for final oocyte maturation may reduce the risk by nearly 80% [23]. There were only two cases of moderate OHSS in our study representing 1.6% of the cohort of patients. Whereas the risk of OHSS is significantly reduced with the GnRH-a trigger for final oocyte maturation, there remains a risk of empty follicle syndrome and our practice is to give HCG trigger if there are than fifteen follicles $\geq$15 mm and oestradiol level is not higher than 15,000 pmol/L [9,24]. Equally as important is the risk of poor response resulting in cycle cancellation or no oocytes/ embryos available for cryopreservation. In a large dataset of 52,676 routine IVF cycles, 6.7% were cancelled due to poor ovarian response [25]. This is devastating for a woman who has a singular opportunity for fertility preservation. In our study, four women (3.1%) who underwent ovarian stimulation had no oocytes or embryos available to freeze due to poor response to ovarian stimulation. While fertility preservation with oocyte or creating embryos may be the golden opportunity for a woman to have biological children, realistic counselling and informed decision making, including information about other available methods should be at the centre-stage of the fertility consultation. Using accurate biomarkers of ovarian reserve such as antral follicle count or AMH will inform women of the risk of anticipated poor response, however, this ought not be grounds to deny treatment [25]. We were unable to establish the number of women who may have considered other options such as ovarian tissue cryo-preservation or use of GnRH-agonist co-treatment as the unit is a standalone referral centre for assisted reproductive treatment.

Women diagnosed with cancer hope for long-term survival and consider starting a family in future [26]. This is mainly due to early diagnosis and improvement in treatment protocols witnessed in recent times [5]. It is reassuring also to note that pregnancy does not affect the prognosis after cancer treatment [27,28]. Experience about the use of cryopreserved oocytes or embryos in women with cancer is limited, however, initial case reports are reassuring with good outcomes [28]. Generally, the interval from end of cancer treatment to attempting pregnancy should be a balance between the end of fetal exposure to anticancer drugs and the beginning of the lowest risk period for disease recurrence. Whereas there is reassuring evidence to suggest women with breast cancer embarking on pregnancy do not have a worse prognosis, still concerns in breast cancer survivors exist, with nearly 30% choosing pregnancy termination [29,30]. Perhaps unsurprisingly, while the majority of women in our study had breast cancer, only two of the fifteen women who attempted a pregnancy had this form of the disease. Additionally, although for various unknown reasons, 12 of the 16 women (75%) who gave consent to discard their cryopreserved embryos had previously had breast cancer.

Our study benefits from a comprehensive database and complete follow-up of all the cryopreserved gametes or embryos due to the requirement of the Human Fertilisation and Embryology Authority that all patients treated in a licensed centre have their treatment and outcome registered with th authority [31]. Although our study demonstrated no statistically significant difference in ovarian stimulation outcomes between the random start and early follicular phase stimulation in antagonist cycles, the conclusions should be taken with caution for lack of power calculation and being retrospective in design. In addition, there may be inherent differences in response to ovarian stimulation between various cancer types which we were unable to demonstrate. For example, it has been shown that women with lymphoma or BRAC-1 gene mutation have reduced ovarian reserve [32]. More studies to determine live birth rate are needed as this is the ultimate outcome for fertility preservation. It would also be important to explore factors surrounding decisions to utilised cryopreserved oocytes or embryos.

In conclusion, we have demonstrated that for women faced with potential gonadotoxic treatment and requiring urgent fertility preservation, ovarian stimulation using the antagonist protocol and started randomly has similar ovarian response and outcomes regardless of the timing in the menstrual cycle. The goal of fertility preservation is to achieve a live birth outcome; therefore, more studies are needed urgently to inform the scientific community the fate of cryopreserved oocytes or embryos and reasons that behind decisions for use or non-use.

Conflict of interest

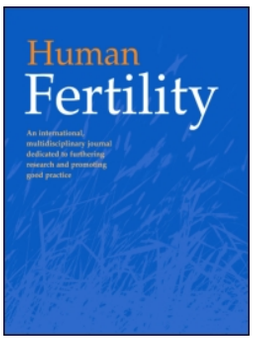
The authors have no conflict of interest to declare.

Acknowledgement

The authors wish to acknowledge Ginny Mounce (PhD), for kindly proof-reading the manuscript and offering valuable critique.

References

[1] Schumacher B., Grover N, Mesen T, Steiner A, Mersereau J. Modeling of live-birth rates and cost-effectiveness of oocyte cryopreservation for cancer patients prior to high- and low-risk gonadotoxic chemotherapy. Hum Reprod 2017;32:2049–55.
[2] Partridge AH, Gelber S, Peppercorn J, Sampson E, Knudsen K, Laufer M, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol 2004;22:4174–83.
[3] Fertility preservation and reproduction in patients facing gonadotoxic therapies: a committee opinion. Fertil Steril 2013;100:1224–31.
[4] Conte B, Poggio F, Del Mastro L. Luteininzing hormone releasing hormones analogs in combination with tamoxifen for the adjuvant treatment of premenopausal women with hormone receptor positive breast cancer. Expert Opin Pharmacother 2017;18:1357–62.
[5] Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917–31.
[6] Donnez J, Dolmans MM. Fertility preservation in women. Nat Rev Endocrinol 2013;9:735–49.
[7] Farquhar C, Rombauts L, Kremer JA, Lethaby A, Ayeleke RO. Oral contraceptive pill, progestogen or oestrogen pretreatment for ovarian stimulation protocols for women undergoing assisted reproductive techniques. Cochrane Database Syst Rev 2017;5:Cd006109.
[8] Siristatidis CS, Gibreel A, Basios G, Maheshwari A, Bhattacharya S. Gonadotrophin-releasing hormone agonist protocols for pituitary suppression in assisted reproduction. Cochrane Database Syst Rev 2015;Cd006919.
[9] Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2016;4:Cd001750.
[10] Turnbull KE, Braden AWH, Mattner PE. The pattern of follicular growth and atresia in the ovine ovary. Aust J Biol Sci 1977;30:229–42.
[11] Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update 2012;18:73–91.
[12] Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. Biol Reprod 2003;69:1023–31.
[13] Sonmezer M, Turkcuoglu I, Coskun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril 2011;95:2125 e9-e1.
[14] Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril 2013;100:1673–80.
[15] von Wolff M, Thaler CJ, Frambach T, Zeeb C, Lawrenz B, Popovici RM, et al. Ovarian stimulation to cryopreserve fertilized oocytes in cancer patients can be started in the luteal phase. Fertil Steril 2009;92:1360–5.
[16] Anderson RA, Kinniburgh D, Baird DT. Preliminary experience of the use of a gonadotrophin-releasing hormone antagonist in ovulation induction/in-vitro fertilization prior to cancer treatment. Hum Reprod 1999;14:2665–8.
[17] Bedoschi GM, de Albuquerque FO, Ferriani RA, Navarro PA. Ovarian stimulation during the luteal phase for fertility preservation of cancer patients: case reports and review of the literature. J Assist Reprod Genet 2010;27:491–4.
[18] Simi G, Obino ME, Casarosa E, Litta P, Artini PG, Cela V. Different stimulation protocols for oocyte cryopreservation in oncological patients: a retrospective analysis of single university centre. Gynecol Endocrinol 2015;31:966–70.
[19] Kim JH, Kim SK, Lee HJ, Lee JR, Jee BC, Suh CS, et al. Efficacy of random-start controlled ovarian stimulation in cancer patients. J Korean Med Sci 2015;30:290–5.
[20] Kasum M, Šimunić V, Orešković S, Beketić-Orešković L. Fertility preservation with ovarian stimulation protocols prior to cancer treatment. Gynecol Endocrinol 2014;30:182–6.
[21] Cakmak H, Rosen MP. Ovarian stimulation in cancer patients. Fertil Steril 2013;99:1476–84.
[22] Practice Committee of the American Society for Reproductive Medicine. Prevention and treatment of moderate and severe ovarian hyperstimulation syndrome: a guideline. Fertil Steril 2016;106:1634–47.
[23] Youssef MAFM, Van der Veen F, Al-Inany HG, Mochtar MH, Griesinger G, Nagi Mohesen M, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev 2014(10)CD008046, doi:http://dx.doi.org/10.1002/14651858.CD008046.pub4.
[24] Revelli A, Carosso A, Grassi G, Gennarelli G, Canosa S, Benedetto C. Empty follicle syndrome revisited: definition, incidence, aetiology, early diagnosis and treatment. Reprod Biomed Online 2017;35:132–8.
[25] La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. Hum Reprod Update 2014;20:124–40.
[26] Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer 2012;118:1710–7.
[27] Stensheim H, Cvancarova M, Moller B, Fossa SD. Pregnancy after adolescent and adult cancer: a population-based matched cohort study. Int J Cancer 2011;129:1225–36.
[28] Peccatori FA, Azim [91_TD$DIFF]Jr HA, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, et al. Cancer, pregnancy and fertility: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24(Suppl. 6):vi160–70.
[29] Ives A, Saunders C, Bulsara M, Semmens J. Pregnancy after breast cancer: population based study. Bmj 2007;334:194.
[30] Azim Jr. HA, Santoro L, Pavlidis N, Gelber S, Kroman N, Azim H, et al. Safety of pregnancy following breast cancer diagnosis: a meta-analysis of 14 studies. Eur J Cancer 2011;47:74–83.
[31] Code of practice. 4th ed. London: Human Fertilisation & Embryology Authority; 1998.
[32] Friedler S, Koc O, Gidoni Y, Raziel A, Ron-El R. Ovarian response to stimulation for fertility preservation in women with malignant disease: a systematic review and meta-analysis. Fertil Steril 2012;97:125–33.



Taylor & Francis
Taylor & Francis Group

Human Fertility

an international, multidisciplinary journal dedicated to furthering research and promoting good practice

ISSN: 1464-7273 (Print) 1742-8149 (Online) Journal homepage: https://www.tandfonline.com/loi/ihuf20

Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers

Jessica Subira, Jo Craig, Karen Turner, Aysha Bevan, Eric Ohuma, Enda McVeigh, Tim Child & Muhammad Fatum

To cite this article: Jessica Subira, Jo Craig, Karen Turner, Aysha Bevan, Eric Ohuma, Enda McVeigh, Tim Child & Muhammad Fatum (2016) Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers, Human Fertility, 19:4, 254-261, DOI: 10.1080/14647273.2016.1223357

To link to this article: https://doi.org/10.1080/14647273.2016.1223357

HUMAN FERTILITY, 2016
VOL. 19, NO. 4, 254–261
http://dx.doi.org/10.1080/14647273.2016.1223357

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers

Jessica Subira[a], Jo Craig[a], Karen Turner[a], Aysha Bevan[a], Eric Ohuma[b], Enda McVeigh[a], Tim Child[a] and Muhammad Fatum[a]

[a]Nuffield Department Obstetrics and Gynaecology, Oxford Fertility Unit, Institute of Reproductive Sciences, University of Oxford, Oxford, UK; [b]Nuffield Department Obstetrics and Gynaecology, University of Oxford, Oxford, UK

ABSTRACT

Debate continues over which morphological parameter is most important in selecting blastocysts for transfer. We aimed to investigate which parameter more accurately predicts the occurrence of a live birth by designing a retrospective cohort study of 1084 fresh elective single blastocyst transfers. Primary outcome was live birth rate (LBR) and secondary outcomes were implantation, clinical pregnancy and early pregnancy loss rates. Blastocyst expansion and inner cell mass (ICM), but not trophoectoderm, were associated with LBR in the definitive multivariable regression analysis. When ICM grade dropped from A to C the likelihood of achieving a live birth was reduced by 55% (OR= 0.45, 95% CI 0.26-0.79, $p = 0.005$). These results were similar for clinical pregnancy rates. Early pregnancy loss rates of embryos with ICM grade C were more than double (38.0%) compared to those of grades A (15.95%) and B (17.17%, $p = 0.002$). The transfer of an embryo with an optimal inner cell mass reduces early pregnancy loss and increases the likelihood of a live birth. We did not find any significant association between trophectoderm and LBR in the multivariable analysis in contrast with recent studies.

ARTICLE HISTORY
Received 14 July 2015
Accepted 18 November 2015

KEYWORDS
Blastocyst; pregnancy and live-birth rates; trophectoderm

Introduction

Blastocyst culture is a worldwide accepted technique that improves implantation and pregnancy rates by selecting the most viable embryo (Gardner et al., 1998; Veeck et al., 2004). In addition, blastocyst transfer is argued to improve synchronicity between embryo and endometrium by mimicking what would happen within a natural conception. Based on these facts, the possibility of elective single blastocyst transfer became a reality in the last decade allowing the maintenance of high pregnancy rates while keeping the risk of multiple pregnancies to a minimum in selected patients.

Morphological blastocyst grading system proposed by Gardner and Schoolcraft (1999) has been repeatedly validated and remains the most accepted and used morphological method to select the blastocyst(s) with the higher potential of implantation throughout the IVF community (Balaban et al., 2000; Gardner, Lane, Stevens, Schlenker, & Schoolcraft, 2000; Gardner et al., 2004). This system relies on three morphological features within the blastocyst development including the grade of expansion, the inner cell mass (ICM) and the trophectoderm (TE). Each blastocyst is graded according to this system leading to a number from one to six (degree of expansion) followed by two letters (A, B or C) to score ICM and TE, respectively, based on the size and compactness of its cells (Gardner et al., 1998).

With more uniform consensus about the critical importance of blastocyst expansion on outcome, several studies have tried to identify the individual contribution of each, ICM and TE grades, to the implantation potential of blastocysts aiming to facilitate even more selection of similar grade blastocysts. However, the conclusions that can be drawn from these studies remain, to some extent, contradictory, with a good number of recent studies pointing in the direction of the TE as the strongest predictor of clinical pregnancy. Clearly, no consensus has yet been achieved on this matter. Therefore, there is still a need for more evidence on this topic, as the question remains unanswered.

To add evidence to this ongoing debate, we designed a retrospective cohort study to further inves-

CONTACT Jessica Subira jessica.subira@gmail.com Nuffield Department Obstetrics and Gynaecology, Oxford Fertility Unit, Institute of Reproductive Sciences, 8000 Alec Issigonis Way, Oxford Business Park North, OX4 2HW. Oxford, UK

tigate the correlation between blastocyst morphology parameters and live birth and clinical pregnancy rates as well as the individual contribution attributable to each parameter.

Materials and methods

Study design and patients

The study obtained ethical approval by the Institutional review board of Oxford University (MSD-IDREC-C1-2014-055). From January 2009 to June 2012, 1084 single fresh blastocyst transfer cycles were performed at the Oxford Fertility Unit. All patients were between 20 and 38 years of age, as above this age a double embryo transfer is routinely recommended to all patients according to our protocols. We included all grades of expansion but we did not include blastocyst transfers on day 6 of development or frozen cycles for consistency as differential endometrial and embryological factors could have interfered with the outcome. There were no other exclusion criteria based upon clinical characteristics. Thus, in total, 1084 patients with a single fresh blastocyst transfer were included for analyses.

Stimulation protocol

A long GnRH agonist protocol was used for all patients apart from those with a diagnosis of polycystic ovarian syndrome (PCOS). For these patients, a short GnRH antagonist protocol was used to reduce the risk of ovarian hyperstimulation syndrome (OHSS). Briefly, patients were started on midluteal phase of previous cycle with nasal nafarelin acetate 800 mcg a day (Synarel®, Pfizer, Sandwich, UK) or buserelin 500 mcg daily administered subcutaneously (Suprefact®, Sanofi, Guildford, UK) which were continued until the day of human chorionic gonadotropin (HCG) after reduction to 400 mcg or 250 mcg a day, respectively, once down-regulation was achieved. The daily gonadotropin administered was either human menopausal gonadotropin (Menopur®, Ferring Pharmaceuticals, West Drayton, UK) or recombinant FSH (Gonal-f®, Merck Serono, Feltham, UK). A fixed GnRH Antagonist protocol was used with the introduction of Cetrorelix 250 mcg a day subcutaneously (Cetrotide®, Merck Serono, Feltham, UK) on day 4 of controlled ovarian stimulation (COS).

Serial ultrasound scans to track follicular growth were performed until at least 3 follicles $\geq$18 mm were observed, when final oocyte maturation was achieved with choriogonadotrofin alfa 6500 IU subcutaneously (Ovitrelle®, Merck Serono, Feltham, UK). The oocyte retrieval was performed 35 h later transvaginally under conscious sedation and ultrasound guidance. Oocytes were either inseminated or microinjected according to sperm parameters and following standardized protocols. Fertilization checks occurred 15-18 h later.

Embryo culture and blastocyst grading

Embryos were initially cultured in cleavage medium (Sydney IVF cleavage medium, K-SICM-100, Cook®) before being moved to blastocyst medium on day 3 (Sydney IVF blastocyst medium, K-SIBM-50, Cook®). Embryos were cultured individually in 50 µl drops, normally with 4 embryos per dish and 2 wash drops. Oxygen and CO_2 concentration were 5 and 6%, respectively.

Embryo development was assessed on day 2 and again on day 3 after fertilization. Culture to and embryo transfer on Day 5 required $\geq$3 embryos to be at the 7–8 cell stage, normally with a combined score for blastomere size and fragmentation of $\geq$6 according to the Cleavage Stage Grading System (Cutting et al., 2008). The classification of Gardner and Schoolcraft (1999) was used to classify the blastocysts. All our embryologists are trained and use this classification on the daily clinical practice. Briefly, the expansion stage was assessed as one of the following: (1) an early blastocyst, blastocoele being less than half volume of that of the embryo; (2) a blastocyst with a blastocoele whose volume is half of, or greater than half of that of the embryo; (3) a full blastocyst with a blastocoele completely filling the embryo; (4) an expanded blastocyst with a blastocoele volume larger than that of the full blastocyst, with a thinning zona; (5) a hatching blastocyst with the TE starting to herniate through the zona; and (6) a hatched blastocyst, in which the blastocyst has completely escaped from the zona. For blastocysts with expansion stage 3 or above, ICM grade and TE grade were evaluated. The ICM was assessed as one of the following: (A) tightly packed, many cells; (B) loosely grouped, several cells; and (C) very few cells. The TE was assessed as one of the following: (a) many cells forming a cohesive epithelium; (b) few cells forming a loose epithelium; and (c) very few, large cells.

Embryos were selected for transfer prioritising expansion above the other parameters (expansion grade $\geq$3). When multiple expanded blastocysts were available, a combination of the ICM and TE grades was used to select the embryo (Aa > Ab > Ba > Bb> …). If there were multiple good quality blastocysts with similar grades then the day 3 grade and the early cleavage

information was taken into consideration to make the final decision.

Embryo transfer and pregnancy testing

Ultrasound-guided embryo transfer was performed on Day 5 of embryo development. Luteal phase support was started on the day after oocyte retrieval with 400 mg/12 h of progesterone administered in the form of vaginal pessaries (Cyclogest®, Actavis, Barnstaple, UK) and continued until a urinary pregnancy test was performed.

The urinary pregnancy test was provided to the patients who were asked to perform it 11 days after the embryo transfer and telephone the unit to communicate the results. If positive, an ultrasound scan to evaluate viability was arranged at 6 and 8 weeks of gestation. For ongoing pregnancies, patients were discharged at 8 weeks of pregnancy after confirming viability with an ultrasound scan and were asked to contact the unit with the final outcome of the pregnancy as required by the Human Fertilisation and Embryology Authority.

Outcomes

The primary outcome measured was live birth rate, defined as the birth of a live new-born above 24 weeks of pregnancy. Secondary outcomes included implantation and clinical pregnancy rates; this latter defined by the presence of fetal heart activity on ultrasound scan at 7 weeks of gestation. Implantation was defined by a positive pregnancy test (βHCG >25 mIU/L in urine). Early pregnancy loss was defined as the loss of the pregnancy before the diagnosis of fetal heart activity via ultrasound assessment. Clinical and treatment related characteristics were recorded for all patients including female age, BMI, infertility diagnosis, type of controlled ovarian stimulation (COS) protocol used, total dose of gonadotropins, number of eggs retrieved and fertilization rate.

Statistical analysis

Data were checked for completeness and missing values and data explorations were performed graphically using scatter, histograms, and box plots and also by running frequency checks and descriptive statistics. A Chi-square test and Fisher's exact test (when the total per cell is less than 5) were used where appropriate to assess associations between any two important variables. The proportions test was used to evaluate differences between two categories. Associations between live birth rate, implantation rate, clinical pregnancy rate, and early pregnancy loss rate as outcomes and embryo stage, inner cell mass grade, and trophectoderm grade as predictors were performed separately in a univariable logistic model. Further, for each of the outcomes, we performed a multivariable logistic regression analyses adjusting for embryo stage, inner cell mass grade, trophectoderm grade and maternal age. All these factors were first considered in a univariable logistic model and only variables that were significant at the 5% level were further considered and included in the multivariable logistic model. All statistical significance was assessed at the 5% level of significance (p value <0.05). All analyses were analysed using STATA 12 (StataCorp, College Station, TX).

Results

A total of 1084 patients were included in the analysis and the median age of the study population was 33.3 years (IQR: 31.0–35.3) and median BMI of 23.0 (IQR: 21.0–25.5). The median number of eggs retrieved was 13 (IQR: 9–17), fertilization rate was 75% (IQR: 63.6–85.7) and median total number of embryos obtained was 8 (IQR: 6-11). Live birth rate was 45.94% with only 0.92% of live births related to twin pregnancies and there were no high-order multiple pregnancies. The overall implantation rate for the cohort was 64.02% and the overall clinical pregnancy rate was 51.66% with 15 multiple pregnancies among these (2.68%). Of those who had a successful implantation, 19.31% experienced an early pregnancy loss. There were 87 (12.54%) miscarriages after the diagnosis of clinical pregnancy (fetal cardiac activity), 3 ectopic pregnancies (0.43%), 2 stillbirths (0.28%) and 7 terminations related to fetal pathology (1%).

Table 1 compares the baseline and treatment characteristics of patients who achieved a live birth and patients who did not. There were no significant differences among them. Figure 1 shows the number of embryo transfers performed according to blastocyst degree of expansion. There were no embryo transfers of hatched blastocysts (grade 6) as we did not include embryo transfers performed on day 6 of development. A live birth was 3.4 (OR = 3.48, 95% CI 1.15–10.50, $p = 0.027$) and 2.5 (OR = 2.58, 95% CI 1.24–5.35, $p = 0.011$) times more likely to occur for hatching embryos and fully expanded blastocysts, respectively, when compared to early blastocysts in a univariable logistic regression. Regarding ICM, grade C resulted in a 60% less likelihood of a live birth when compared to grade A (OR 0.39, 95% CI: 0.23–0.66, $p = 0.001$). The reduction in the ICM from A to B appears not to affect

Table 1. Summary of baseline and treatment characteristics in patients achieving a live birth and those not achieving a live birth.

	No live birth	Live birth	*p* Value
Patient and cycle characteristics[a]			
Age (years)	32.99	32.75	0.21
BMI (kg/m^2)	23.57	23.59	0.92
Total number of gonadotropins units used	1915.26	2028.14	0.59
Total number of eggs collected	13.56	13.86	0.43
Type of protocol used[b]			
Antagonist protocol	32	18	
Long agonist protocol	554	480	0.15
Infertility diagnosis[b]			
Combined male/female infertility	107	96	
Endometriosis	23	27	
Previous failed donor insemination	2	1	
Male factor	141	120	
Female infertility: multiple diagnosis	47	45	0.95
PCOS	72	58	
Tubal pathology	54	40	
Unexplained infertility	124	100	
Other	16	11	
Cycle number[c]			
1	471	396	
2	96	88	
3	15	12	0.89
4	4	2	
TOTAL	N = 586	N = 498	

Statistical tests used were:
[a]Student's *t*-test.
[b]Chi-square.
[c]Fisher's exact.

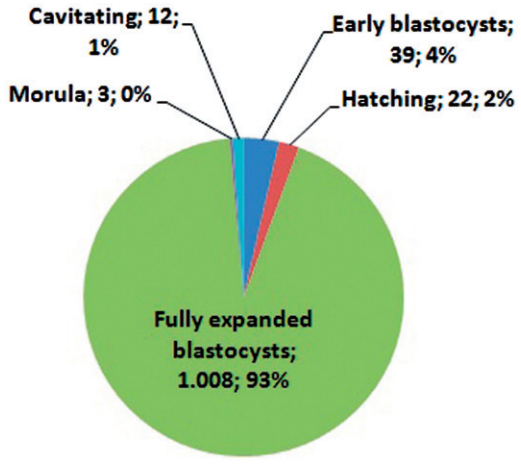


Figure 1. Distribution of embryo transfers performed according to embryo stage.

the live birth in a significant manner according to our study (OR 0.78, 95% CI 0.58-1.04, $p = 0.099$). This is significant though when a more important drop in the quality of the ICM occurs (from A to C). For TE, the association with LBR was significant for TE grade c (OR 0.53, 95% CI: 0.33–0.84, $p = 0.007$) when compared to grade a. Similar results were observed for CPR (Table 2).

We performed a multivariable logistic regression to confirm these preliminary results in two separate models. On the first one and after adjusting by age, LBR according to blastocyst expansion was analyzed (Table 2). The association remained significant for all grades of expansion, except for cavitating or grade 1 embryos, probably because the number of cases included was comparatively small ($n = 12$) compared to the other groups of expansion. We then designed a second model to investigate ICM and TE after adjusting by age. For LBR, the only predictor that remained significant was ICM grade C (OR= 0.45, 95% CI: 0.26–0.79, $p = 0.005$). Thus, the inner cell mass grade C was the only one that showed a statistically significant association with LBR and CPR when compared to grade A, by reducing the likelihood of a live birth occurring by 55%. There were no significant associations between TE and LBR or CPR (Table 2). Of note, because of multicollinearity, derived from the intrinsic association between blastocyst expansion and ICM and TE grades, it was not possible to introduce all three parameters on the same multivariable regression model.

Replicating the interesting analysis performed by Hill et al. (2013) we created different categories of combinations of grades between ICM and TE. The most prevalent combination was Bb accounting for 529 transfers (51.36% of all the transfers with grade 3 or higher). Preliminary analysis did suggest an association between combinations of grades between ICM and TE (Aa, Ab, Ba …) with LBR (Chi-square $p = 0.011$). There was no statistical difference with a drop in the TE grade from a to b when the ICM remained unchanged ($p = 0.710$). A drop in the ICM grade from A to B with the TE remaining unchanged also showed no effect ($p = 0.644$) on the LBR. The results were similar for CPR and IR.

Early pregnancy loss rates were found to be significantly correlated with embryo stage (p value = 0.001) and ICM grade (p value = 0.002) but not with TE grade (p value = 0.668). Early pregnancy loss rates of embryos with grade C ICM were more than double (38.00%) compared to those of grades A (15.95%) and B (17.17%, $p = 0.002$).

Discussion

Our study confirms an association between blastocyst expansion and live birth rates in the multivariable analysis, which has been repeatedly corroborated on all the studies published about the topic (Gardner et al., 2000; Shapiro, Harris, & Richter, 2000; Thompson, Onwubalili, Brown, Jindal, & McGovern, 2013; Van den Abbeel et al., 2013; Yoon, Yoon, Son, Im, & Lim, 2001). In contrast with the most recent published studies

Table 2. Univariable regression analysis (live birth and clinical pregnancy rates) and multivariable regression analysis (live birth rate).

	No live birth	Live birth	LBR OR (95% CI, *p*) Univariable	No clinical pregnancy	Clinical pregnancy	CPR OR (95% CI, *p*) Univariable	No live birth	Live birth	LBR OR (95% CI, *p*) Multivariable[b]
Blastocyst stage									
Early blastocysts	29	10	1	29	10	1	29	10	1
Hatching blastocysts	10	12	3.48 (1.15–10.5, $p = 0.03$)	6	16	7.73 (2.37–25.21, $p < 0.01$)	10	12	3.45 (1.14–10.44, $p = 0.03$)
Fully expanded	533	475	2.58 (1.25–5.36, $p = 0.01$)	475	533	3.25 (1.57–6.74, $p < 0.01$)	533	475	2.59 (1.25–5.38, $p = 0.01$)
Morula[a]	3	0	–	3	0	–	3	0	–
Cavitating	11	1	0.26 (0.03–2.31, $p = 0.23$)	11	1	0.26 (0.30–2.31, $p = 0.23$)	11	1	0.26 (0.29–2.28, $p = 0.23$)
ICM									
Grade A	113	129	1	105	137	1	113	129	1
Grade B	372	332	0.78 (0.58–1.04, $p = 0.099$)	323	381	0.90 (0.67–1.21, $p = 0.50$)	372	332	0.84 (0.61–1.14, $p = 0.28$)
Grade C	58	26	0.39 (0.23–0.66, $p = 0.001$)	53	31	0.45 (0.27–0.75, $p < 0.01$)	58	26	0.45 (0.25–0.78, $p < 0.01$)
TE									
Grade a	60	71	1	53	78	1	60	71	1
Grade b	378	350	0.78 (0.54–1.14, $p = 0.198$)	336	392	0.79 (0.54–1.15, $p = 0.23$)	378	350	0.87 (0.58–.28, $p = 0.49$)
Grade c	105	66	0.53 (0.33–0.84, $p = 0.007$)	92	79	0.58 (0.36–0.92, $p = 0.02$)	105	66	0.67 (0.40–1.10, $p = 0.12$)

[a]No implantation for morulas ($n = 3$, 0.28%).
[b]Multivariable logistic regression analysis adjusting for embryo stage, inner cell mass grade, trophectoderm grade, and maternal age (two separate multivariable regression analyses were performed as described in the text but results have been presented jointly on this table).

(Ahlstrom, Westin, Reismer, Wikland, & Hardarson, 2011; Hill et al., 2013; Honnma et al., 2012; Thompson et al., 2013), which did not find the ICM to be significant and focused on the TE as main predictor of outcome, we found a strong association between LBR and ICM in the multivariable analysis and none between TE and measured outcomes.

Some authors have published that the degree of expansion is the most predictive factor of implantation (Dokras, Sargent, & Barlow, 1993; Shapiro et al., 2000; Yoon et al., 2001). Others though advocate the quality of the inner cell mass as the most important factor related to implantation potential (Balaban et al., 2000; Richter, Harris, Daneshmand, & Shapiro, 2001; Shapiro, Richter, Harris, & Daneshmand, 2001). In our study, we found a significant association between LBR and ICM in the multivariable analyses.

Recently, three studies have reported similar conclusions identifying an association between the TE morphology and implantation rates (Ahlstrom et al., 2011; Hill et al., 2013; Honnma et al., 2012; Thompson et al., 2013). However, methodological differences among these studies can be argued since the one from Honnma et al. (2012) was performed in frozen blastocysts, hence non-controlled endometrial factors could have played an important role. Other authors have grouped good quality blastocysts under new created categories making the results not comparable with previous studies (Goto et al., 2011).

One of the most recent papers from Hill et al. (2013) analyzing almost 700 single blastocyst transfers concluded that the TE was the strongest predictor of live birth with no apparent contribution of the ICM to the outcomes. Our study, which includes a higher number of cases, did not confirm this association in the multivariable analysis. They also created different categories according to the possible combinations between TE and ICM grade and confirmed the importance of the TE by finding a significant reduction in IR and live birth rate when the TE dropped from a to b. Analogously, we replicated the analysis of these different combinations and did not confirm this reduction in LBR when the TE dropped from a to b.

Thompson et al. (2013) recently published data of more than 3000 cycles retrieved from the Society for Assisted Reproductive Technologies registry showing that blastocyst expansion stage, TE grade and age predict clinical pregnancy and live birth. For Van den Abbeel et al. (2013) in their recent study including over 600 single fresh blastocyst transfers all three parameters correlated with live birth rate in the simple logistic regression analysis. Interestingly in the multivariate logistic regression only the blastocyst

expansion remained a significant predictor. In our study, once multivariable regression was applied only blastocyst expansion and ICM grade showed a significant correlation with live birth, which was especially important for ICM grade C.

We found a significant association between blastocyst expansion and poor ICM grade with early pregnancy loss. In the latter study from Van den Abbeel et al. (2013), they report an association between ICM and early pregnancy loss. This is in keeping with our findings and implies a strong association between a robust ICM and viable pregnancy beyond the initial positive pregnancy test.

The importance of the quality of the ICM is confirmed in this study as one of the most important predictors when it comes to select single blastocysts for transfer among those with similar grades of expansion. Deselecting an embryo with a poor quality ICM (grade C) over TE grade will increase the likelihood of achieving a live birth considerably. This has a plausible biological explanation given that this particular parameter is a reflection of embryo competence and it could be associated with higher rate of aneuploidies, which will result in turn into a higher miscarriage rate (Alfarawati et al., 2011). However, this correlation is not absolute with some aneuploid embryos managing to reach the blastocyst stage (Fragouli et al., 2013). Interestingly and in contrast with the most recent body of literature, TE failed to demonstrate this association in the multivariable analysis, in line with the results from Van den Abbeel et al. (2013). Various arguments could possibly explain this difference. Firstly, blastocyst grading, despite using a standardized score system, is subject to variability to some extent. This means that same embryos may have been given different grades on different studies and even on the same study by different observers making the results not comparable. This highlights the importance of standardization of intra and inter-observer variability and the need for strict internal quality control within centres to promote continuous training thus reducing the impact of subjectivity on treatment and research. Secondly, the study size may have affected the power of this study to detect the difference. We have included more cases than Hill et al. (2013) but fewer than Thompson et al. (2013) and it is not possible to exclude the effect of this on the results.

We acknowledge certain limitations to our study, which should encourage caution on the interpretation of the results. Firstly, the current study is retrospective in nature and so is the case of most of the studies published on this topic. We accept this as a weakness to our study but nevertheless we think that it still provides a good insight as to which blastocyst parameter should be prioritized above others. A second limitation is the small number of non-fully expanded or hatching transfers included ($n = 54$, 5.0%), not providing a complete homogenous group for comparison in reference to blastocyst expansion as a parameter. This is explained by the fact that a good proportion of patients with non-fully expanded blastocysts will not opt for a single transfer approach aware of the known reduction on implantation rates. This could represent a source for selection bias, as cycles with lower quality embryos in which double transfers are performed have not been included. Yet, this proportion is similar to the one reported in other studies and it does not affect the ICM and TE parameters as these are only graded on fully expanded blastocysts, which represent more than 90% of all the transfers. There is no adjustment in the multivariable analysis for BMI or other potential confounders such as number of previous cycles or number of units of gonadotropins used. However, the mean BMI of the cohort was 23.0 and therefore we think it unlikely to have affected the results in any manner. Moreover, there were no significant differences on the BMI or other baseline or treatment characteristics of patients achieving a live birth compared to patients who did not as presented in Table 1. Based on the wide inclusion criteria inherent to the design of our study, patients who had more than one single blastocyst transfer in the period studied were included more than once. Because it is well known that the number of cycles performed can have an impact on success rates, a comparison between patients who achieved a live birth and patients who did not depending on cycle number was performed and it is shown in Table 1. No differences were found attending to cycle number.

As valuable strengths of our study we could underscore the high number of cycles included and the exclusion of frozen cycles and double transfers. The exclusion of frozen cycles relates to the differential endometrial factors that could have impacted on the analyzed outcomes should we have included fresh and frozen cycles. We acknowledge that there is an increasing body of evidence suggesting that the replacement of embryos in frozen cycles appears to be more physiological than the replacement in the context of high oestrogen levels achieved after controlled ovarian stimulation in fresh cycles (Roque et al., 2013). Nevertheless, fresh transfers are still widely used in the majority of IVF centres as the standard approach, especially for first cycles, and consequently it is clinically relevant to analyze the outcomes in this type of cycles.

We conclude that blastocyst expansion and ICM correlate with the chances of achieving a live birth as reported in previous studies. Blastocyst expansion appears to be an unquestionable predictor of outcome as discussed above. Inner cell mass (when a drop to Grade C occurs) stands out clearly among the rest as a deselecting tool of a blastocyst that is unlikely to result in a live birth among those with similar grades of expansion. Therefore, an optimal ICM may reduce the risk of early pregnancy loss and increase the likelihood of a live birth occurring. We think it is too early to assume a benefit of the TE grade above the ICM as a predictor of outcome based on previous studies and we did not find a significant association on this respect. Randomized controlled studies are needed in order to determine which parameter among these should be prioritized in the selection of the single blastocyst for transfer.

Acknowledgments

We would like to thank specially Ginny Mounce for her help and collaboration with the preparation of this article.

Disclosure statement

There was no external funding for the purposes of this study. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Ahlstrom, A., Westin, C., Reismer, E., Wikland, M., & Hardarson, T. (2011). Trophectoderm morphology: An important parameter for predicting live birth after single blastocyst transfer. *Human Reproduction*, *26*, 3289–3296. doi:10.1093/humrep/der325.

Alfarawati, S., Fragouli, E., Colls, P., Stevens, J., Gutierrez-Mateo, C., Schoolcraft, W.B. … Wells, D. (2011). The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. *Fertility and Sterility*, *95*, 520–524. doi:10.1016/j.fertnstert.2010.04.003.

Balaban, B., Urman, B., Sertac, A., Alatas, C., Aksoy, S., & Mercan, R. (2000). Blastocyst quality affects the success of blastocyst-stage embryo transfer. *Fertility and Sterility*, *74*, 282–287. doi:10.1016/S0015-0282(00)00645-2.

Cutting, R., Morroll, D., Roberts, S.A., Pickering, S., & Rutherford, A.; BFS & ACE. (2008). Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. *Human Fertility*, *11*, 131–146. doi:10.1080/14647270802302629.

Dokras, A., Sargent, I.L., & Barlow, D.H. (1993). Human blastocyst grading: an indicator of developmental potential? *Human Reproduction*, *8*, 2119–2127.

Fragouli, E., Alfarawati, S., Spath, K., Jaroudi, S., Sarasa, J., Enciso, M., & Wells, D. (2013). The origin and impact of embryonic aneuploidy. *Human Genetics*, *132*, 1001–1013. doi:10.1007/s00439-013-1309-0.

Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., & Schoolcraft, W.B. (2000). Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertility and Sterility*, *73*, 1155–1158. doi:10.1016/S0015-0282(00)00518-5.

Gardner, D.K., & Schoolcraft, W.B. (1999). *In vitro culture of human blastocysts*. In R. Jansen & D. Mortimer (Eds.), *Towards reproductive certainty: Fertility and genetics beyond 1999* (pp. 378–388). Carnforth, UK: Parthenon Publishing.

Gardner, D.K., Schoolcraft, W.B., Wagley, L., Schlenker, T., Stevens, J., & Hesla, J. (1998). A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Human Reproduction*, *13*, 3434–3440. doi:10.1093/humrep/13.12.3434.

Gardner, D.K., Surrey, E., Minjarez, D., Leitz, A., Stevens, J., & Schoolcraft, W.B. (2004). Single blastocyst transfer: A prospective randomized trial. *Fertility and Sterility*, *81*, 551–555. doi:10.1016/j.fertnstert.2003.07.023.

Goto, S., Kadowaki, T., Tanaka, S., Hashimoto, H., Kokeguchi, S., & Shiotani, M. (2011). Prediction of pregnancy rate by blastocyst morphological score and age, based on 1,488 single frozen-thawed blastocyst transfer cycles. *Fertility and Sterility*, *95*, 948–952. doi:10.1016/j.fertnstert.2010.06.067.

Hill, M.J., Richter, K.S., Heitmann, R.J., Graham, J.R., Tucker, M.J., DeCherney, A.H. … Levens, E.D. (2013). Trophectoderm grade predicts outcomes of single-blastocyst transfers. *Fertility and Sterility*, *99*, 1283–1289 e1281. doi:10.1016/j.fertnstert.2012.12.003.

Honnma, H., Baba, T., Sasaki, M., Hashiba, Y., Ohno, H., Fukunaga, T. … Asada, Y. (2012). Trophectoderm morphology significantly affects the rates of ongoing pregnancy and miscarriage in frozen-thawed single-blastocyst transfer cycle in vitro fertilization. *Fertility and Sterility*, *98*, 361–367. doi:10.1016/j.fertnstert.2012.05.014.

Richter, K.S., Harris, D.C., Daneshmand, S.T., & Shapiro, B.S. (2001). Quantitative grading of a human blastocyst: Optimal inner cell mass size and shape. *Fertility and Sterility*, *76*, 1157–1167. doi:10.1016/S0015-0282(01)02870-9.

Roque, M., Lattes, K., Serra, S., Sola, I., Geber, S., Carreras, R., & Checa, M.A. (2013). Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: A systematic review and meta-analysis. *Fertility and Sterility*, *99*, 156–162. doi:10.1016/j.fertnstert.2012.09.003.

Shapiro, B.S., Harris, D.C., & Richter, K.S. (2000). Predictive value of 72-hour blastomere cell number on blastocyst development and success of subsequent transfer based on the degree of blastocyst development. *Fertility and Sterility*, *73*, 582–586.

Shapiro, B.S., Richter, K.S., Harris, D.C., & Daneshmand, S.T. (2001). A comparison of day 5 and day 6 blastocyst transfers. *Fertility and Sterility*, *73*, 1126–1130. doi:10.1016/S0015-0282(99)00586-5.

Thompson, S.M., Onwubalili, N., Brown, K., Jindal, S.K., & McGovern, P.G. (2013). Blastocyst expansion score and trophectoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer (eSET): A national study. *Journal of Assisted Reproduction and Genetics*, *30*, 1577–1581. doi:10.1007/s10815-013-0100-4.

Van den Abbeel, E., Balaban, B., Ziebe, S., Lundin, K., Cuesta, M.J., Klein, B.M. … Arce, J.C. (2013). Association between blastocyst morphology and outcome of single-blastocyst transfer. *Reproductive Biomedicine Online*, *27*, 353–361. doi:10.1016/j.rbmo.2013.07.006.

Veeck, L.L., Bodine, R., Clarke, R.N., Berrios, R., Libraro, J., Moschini, R.M. … Rosenwaks, Z. (2004). High pregnancy rates can be achieved after freezing and thawing human blastocysts. *Fertility and Sterility*, *82*, 1418–1427. doi:10.1016/j.fertnstert.2004.03.068.

Yoon, H.J., Yoon, S.H., Son, W.Y., Im, K.S., & Lim, J.H. (2001). High implantation and pregnancy rates with transfer of human hatching day 6 blastocysts. *Fertility and Sterility*, *75*, 832–833. doi:10.1016/S0015-0282(00)01797-0.

Unusual presentation of metastatic Crohn's disease

Dear Editor,

We wish to report a very unusual case of Crohn's disease, which manifested in the form of a vulval polyp. A 50-year-old lady presented to the gynaecology outpatients clinic with a history of sudden onset swelling of 2–3 months' duration in the right labium minor. There was not much increase in the size of the swelling after the onset and no history of any pain or abnormal discharge.

She had a past medical history of severe Crohn's disease for the last 15 years. Medical treatment with azathioprine and steroids was unsuccessful at controlling her symptoms. The patient had initially undergone a hemicolectomy and ileo-rectal anastomosis followed by a pancolectomy and ileostomy. She later developed a perianal abscess, which was drained. She had recurrence of the ileitis in the ileostomy stoma leading to an entero-cutaneous fistula for which she had a revision ileostomy. She was then started on Sandostatin injections (20 mg every 3 weeks) and she remains symptom-free until now as far as her bowels are concerned.

On examination, she was systemically well. Abdominal examination revealed a functioning ileostomy. On the right labium minor there was a pedunculated mass of 3–4 cm. No other ulcer or fissure was noted. The patient had an excision biopsy under general anaesthesia. The excision site was closed with Vicryl Rapide and healed with no problems.

Histological examination revealed a fibro-epithelial polyp with an oedematous fibro-vascular core in which there were many non-caseating epithelial granulomata, features representing cutaneous metastatic Crohn's disease (Fig. 1). Review after 6 months revealed a normal looking vulva with no signs of recurrence.

Medical management has been the mainstay of treatment of vulval Crohn's disease as the usual presentation is either vulval ulcer or vulval oedema and the diagnosis is made or at least suspected before histological confirmation of the condition [1]. Oral metronidazole, augmentin, azathioprine and topical steroid creams have all been used with some success to control vulval symptoms.

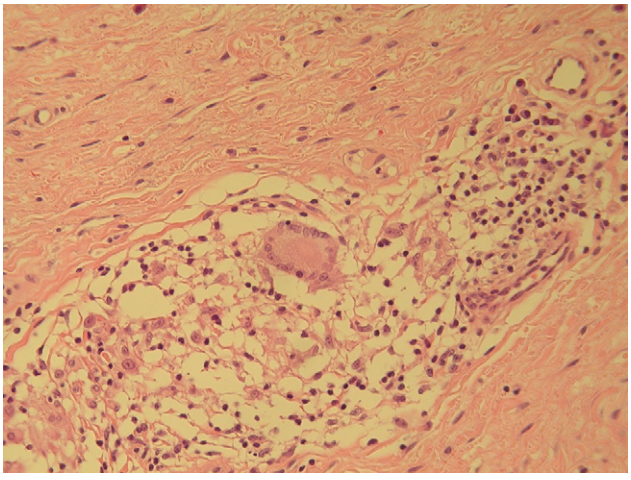


Fig. 1. Histological picture of the polyp showing granuloma in the fibro-vascular core in the centre of the field.

In our case, the diagnosis was not suspected as the patient's intestinal symptoms were well controlled at the time of presentation and it is extremely rare for metastatic Crohn's to be in the form of a polyp. Although the extra-intestinal manifestations run parallel to the intestinal symptoms, metastatic disease can still be present in the absence of any intestinal symptoms. This case showed that diagnosis of Crohn's disease should be considered in cases of vulval polyps. Conservative surgical treatment may be the right way to treat solid lesions [2], although concerns about poor healing and recurrence remain. In our case the wound healing was very good and there is no sign of recurrence so far, although long-term follow-up may prove different.

References

[1] Aviles-Izquierdo JA, Suarez-Fernandez R, Lazaro-Ochaita P, Longo-Imedio I. Metastatic Crohn's disease mimicking genital pyoderma gangrenosum in an HIV patient. Acta Derm Venereol 2005;85(1): 60–2.
[2] Mould TA, Rodgers ME, Burnham WR, Weekes ARl. Metastatic Crohn's disease causing a vulval mass and involving the cervix. Int J STD AIDS 1997;8(7):461–3.

Krithiga Rajagopal Ilangovan*
Richard Yeo
Hull Royal Infirmary, UK

*Corresponding author. Fax: +44 1482 862487
E-mail address: drkrithiga@yahoo.com
(K.R. Ilangovan)

25 February 2006

doi:10.1016/j.ejogrb.2006.12.022

The safety of ultrasound-guided oocyte pick-up in IVF patients with haemostatic disorders

Dear Editor,

As we know, the preoperative assessment of an in vitro fertilization (IVF) patient's haemostatic status and the approach towards those patients having haemostatic defects remains unstudied. To the best of our knowledge, there are no publications in the gynaecologic professional literature dealing with this important topic and its practical applications with IVF patients.

The puncture of the hypervascularized overstimulated ovaries poses a particularly great challenge to haemostatic

Table 1
Patients' characteristics, preparations for OPU and clinical outcomes

	Age	Cause of infertility	Total cycles	Day 3 E2	Day 3 FSH	Disorder	Preoperative preparation	COH protocol	Mean E2	Mean oocyte	Preg.	Preg. outcome
1	28	Male factor NOA	5	141	9.7	F VII deficiency	FFP*3 before OPU	Long	11,044	17	2	NVD*2
2	29	Unexplained	4	43	2.8	VW type B2	F VIII during OPU	Long	3377	8.3	1	NVD
3	32	Unexplained	3	58	3.4	F XI deficiency	FFP*2 before OPU	Long	9960	6.3	3	NVD*2
4	36	Male factor OTA	1	73.4	7.1	FVII deficiency	FFP*2 before OPU	Short	5384	7	1	NVD twins
5	25	Male factor OTA	5	95	3.9	ITP <50,000 plt	IVIG + steroids	Long	6469	12	1	miscarriage
6	40	Male factor OTA	2	10.5	4.3	FVII deficiency	FFP*2 before OPU	Antagonist	3839	6.5	2	NVD*1 miscarriage*1
7	33	Mechanical factor	2	71	4.4	F XI deficiency	FFP*4 before OPU	Long	6991	12	1	O.P.
8	24	Unexplained	1	133	5.9	F XI deficiency	FFP*2 before OPU		12,200	16	1	O.P.

NOA, non-obstructive azospermia; OTA, oligoteratoastenospermia; E2, estradiol pmol/L; FSH, follicle stimulating hormone IU/L; COH, controlled ovarian hyperstimulation; FFP, fresh frozen plasma; IVIG, intravenous immunoglobulin; NVD, normal vaginal delivery; OP, ongoing pregnancy.

competence. Thus, the safety of ovum pick-up in patients with haemostatic disorders is of crucial importance.

During the last 6 years, we reviewed 1800 IVF patient files undergoing IVF-ET of whom 8 (undergoing 23 cycles of oocyte retrieval) were found to have a bleeding tendency disorder. The preoperative haemostatic evaluation, the preparation of the patients before ovum pick-up (OPU), and the clinical course of ovum retrieval and the post-operative course were evaluated. Further evaluation, preparation and medication before OPU of patients with abnormal results were performed as needed by consultant haematologist. Patient's characteristics are shown in Table 1.

In two patients, haemostatic tendency was known before admission to IVF program due to their bleeding histories. In six cases the bleeding tendency was revealed by routine blood tests and none of these patients had a suggestive history. All women were treated preoperatively and some also immediately postoperatively by transfusion of fresh frozen plasma (FFP), concentrated preparations of the deficient clotting factor, intravenous immunoglobulins (IVIG) and steroids (Table 1). All patients were discharged after overnight observation without related complications. In addition, there was no significant influence in regard to IVF outcome.

Transvaginal ultrasound guided ovum pick-up is the method of choice for OPU among most IVF units. The procedure, which involves puncture, then aspiration of ovarian follicles via vaginal route, unavoidably results in damage to the fine vascular haemostasis on the ovarian surface and theca interna layer. Dessole et al., found that the estimated blood loss 24 h after OPU was 230 mL [1]. Severe life-threatening bleeding complications may rarely occur [2].

The mainstay for recognition of a clinically significant bleeding state is still the clinical, personal and familial histories and the physical examination. Several levels of probability for the existence of a bleeding disorder were outlined by Rapaport offering some preoperative laboratory tests for the confirmation of such a clinical suspicion [3]. Although these broad guidelines did not attain widespread clinical use, they add to other publications showing the importance of a detailed history in the presurgical assessment of potential haemostatic problems. In fact, the value of routine preoperative haemostatic screening tests for asymptomatic patients has not been shown [4].

In our study, six of eight patients had negative personal or familial bleeding history and were discovered only by routine preoperative tests. Preoperative evaluation of haemostatic defects should be performed in patients coming from a high-risk background even if there is a negative history of bleeding tendency (i.e. Ashkenazi Jews in our study) [5].

Ovum pick-up should be considered as one posing a particularly great challenge to haemostatic competence and consequently of great risk to undiagnosed patients.

From this observational study, we can infer that with proper preparation aiming at restoration of the haemostatic competence by specific treatment, this group of patients with haemostatic defects could be offered ovum pick-up with good margins of safety.

We propose that this workup should include routine complete blood count (CBC) and PT/PTT tests for high-risk populations with known common inherited bleeding problems. A thorough evaluation of this important topic needs to be further studied before clear-cut and general guidelines can be formed.

References

[1] Dessole S, Rubattu G, Ambrosini G, Miele M, Nardelli GB, Cherchi PL. Blood loss following noncomplicated transvaginal oocyte retrieval for in vitro fertilization. Fertil Steril 2001;76:205–6.
[2] Dicker D, Ashkenazi J, Feldberg D, Levy T, Dekel A, Ben-Rafael Z. Severe abdominal complications after transvaginal ultrasonographically guided retrieval of oocytes for in vitro fertilization and embryo transfer. Fertil Steril 1993;59:1313–5.
[3] Rapaport SI. Preoperative hemostatic evaluation: which tests, if any? Blood 1983;61:229–31.
[4] Houry S, Georgeac C, Hay JM, Fingerhut A, Boudet MJ. A prospective multicenter evaluation of preoperative hemostatic screening tests. The French Associations for Surgical Research. Am J Surg 1995;170:19–23.

[5] Asaki R, Chung DW, Davie EW, Seligsohn U. Factor XI deficiency in Ashkenazi Jews in Israel. N Engl J Med 1991;325:153–8.

Muhammad Fatum*
Cuneyt Ozcan
Alex Simon
Aby Lewin
Neri Laufer
IVF Unit, Department of Obstetrics and Gynecology, Hadassah Hebrew University Hospital, Ein-Kerem, P.O. Box 12000, Jerusalem 91120, Israel, Jerusalem, Israel.

*Corresponding author. Tel.: +972 2 677 6424; fax: +972 2 643 3337
E-mail address: fatum@md.huji.ac.il (M. Fatum)

17 November 2004

doi:10.1016/j.ejogrb.2007.01.010

Gynecological Endocrinology

ISSN: 0951-3590 (Print) 1473-0766 (Online) Journal homepage: https://www.tandfonline.com/loi/igye20

Simplified artificial endometrial preparation, using oral estradiol and novel vaginal progesterone tablets: a prospective randomized study

A. Lewin, G. Pisov, R. Turgeman, M. Fatum, Y. Shufaro, A. Simon, N. Laufer, A. Revel, B. Reubinoff & A. Safran

To cite this article: A. Lewin, G. Pisov, R. Turgeman, M. Fatum, Y. Shufaro, A. Simon, N. Laufer, A. Revel, B. Reubinoff & A. Safran (2002) Simplified artificial endometrial preparation, using oral estradiol and novel vaginal progesterone tablets: a prospective randomized study, Gynecological Endocrinology, 16:2, 131-136, DOI: 10.1080/gye.16.2.131.136

To link to this article: https://doi.org/10.1080/gye.16.2.131.136

Published online: 07 Jul 2009.

Gynecol Endocrinol 2002;16:131–136

Simplified artificial endometrial preparation, using oral estradiol and novel vaginal progesterone tablets: a prospective randomized study

A. Lewin, G. Pisov, R. Turgeman, M. Fatum, Y. Shufaro, A. Simon, N. Laufer, A. Revel, B. Reubinoff and A. Safran*

In Vitro Fertilization Unit, Department of Obstetrics and Gynecology; *Department of Pathology, Hadassah University Hospital, Ein-Kerem, Jerusalem, Israel

Key words: ARTIFICIAL ENDOMETRIAL PREPARATION, ESTRADIOL, VAGINAL PROGESTERONE

ABSTRACT

There are various successful protocols for artificial endometrial preparation, comprising induction of endometrial proliferation with estrogens and secretory transformation with progestins. The aim of this prospective randomized study was to evaluate a simplified approach for endometrial preparation, comparing two constant doses of oral estradiol combined with a novel low-dose vaginal natural progesterone preparation (100 mg Endometrin® tablets). Twenty-nine patients were enrolled in the study and divided randomly into two groups. Both groups received oral estradiol tablets from the beginning of menstruation, group A (15 patients) receiving 4 mg/day divided into two doses of 2 mg each, and group B (14 patients) receiving 6 mg/day divided into three doses. Serum estradiol and progesterone and sonographic thickness of the endometrium were measured on the 1st day of menstruation and on the 6th, 11th, 16th and 21st days of the artificial cycle. Following the first 12 days of estradiol priming, with an endometrial thickness of ≥ 8 mm, Endometrin vaginal tablets 100 mg were added twice a day for 10 days. On the 21st cycle day, an endometrial biopsy was taken from all patients using Pipelle®.

In all 29 patients, appropriate changes in estradiol, progesterone and endometrial thickness were observed. Estradiol levels were significantly higher in the 6 mg/day group on days 6 and 11, but no significant difference was noted in serum progesterone level and endometrial thickness between groups. Histological evaluation of endometrial biopsies, on the 21st day, revealed adequate late-secretory endometrium in 14/15 (93.3%) patients of group A and in 13/14 (92.9%) patients of group B. In conclusion, our results demonstrate that an appropriate endometrial secretory transformation may be induced using an economical regimen of fixed low-dose oral estradiol (4 mg/day) and low-dose vaginal progesterone tablets (Endometrin 100 mg twice daily).

INTRODUCTION

Since the first successful attempts to establish normal endometrial development and maintain pregnancies in patients with absent or non-functioning ovaries[1,2], it has been well documented that exogenous administration of estrogens and progestogens may induce an artificial

Correspondence: Dr A. Lewin, IVF Unit, Hadassah University Hospital, Ein-Kerem, Jerusalem, Israel

endometrial preparation for embryo transfer, both in ovarian failure patients and in normally ovulating patients scheduled for the transfer of frozen–thawed embryos. Various protocols for artificial endometrial preparation have been studied, including the use of a variable estradiol protocol combined with a fixed intramuscular progestin dose; the use of a fixed oral estradiol dose with a fixed intramuscular progestin dose; the use of different oral estradiol doses and a fixed dose of oral progestins; estradiol implants and intramuscular progestins; and oral estradiol and different progestin preparations. Also, the efficacy of transdermal estradiol and vaginal progestogen gel, and of combining gonadotropin-releasing hormone analogs (GnRHa) in artificial cycles of recipients with functioning ovaries prepared for oocyte donation or of frozen–thawed embryos, and the role of spontaneous and induced cycles have been assessed[2–11]. Clearly, some protocols are less convenient than others, with regard to the use of artificial agonadism with GnRHa for priming the artificial cycle, the use of variable estradiol doses or elevated amounts of estradiol during the proliferative phase, and the use of an intramuscular progestin or multiple vaginal progestogen applications. The aim of this prospective randomized study, therefore, was to evaluate a simplified protocol, comparing the effect of two fixed doses of oral estradiol combined with a new low-dose vaginal natural progesterone preparation (100 mg Endometrin® tablets) on serum estradiol and progesterone levels, endometrial thickness and endometrial histological dating.

MATERIALS AND METHODS

Study design

Twenty-nine patients expecting frozen–thawed embryo transfer in the *in vitro* fertilization (IVF) program of the Hadassah University Hospital, Ein-Kerem, were enrolled in this study. The institutional Helsinki committee approved the study, and informed consent was obtained from each patient. Patients were divided prospectively and randomly into two groups. Both groups received oral Estrofem® 2 mg tablets (Novo, Denmark) from the beginning of menstruation, group A (15 patients) receiving 4 mg/day divided into two doses of 2 mg each, and group B (14 patients) receiving 6 mg/day divided into three doses. The mean age of patients was similar in both groups (32.5 ± 5.8 years in group A and 32.1 ± 6.3 years in group B). On the 11th day of estradiol priming, with an endometrial thickness of ≥ 8 mm, Endometrin 100 mg vaginal tablets (Florish, Israel) were added twice daily.

Hormonal and endometrial monitoring

Blood samples for immunoassay of serum estradiol levels (measured in pmol/l) and serum progesterone levels (measured in nmol/l) were collected, and endometrial thickness was sonographically measured starting on the 1st day of menstruation and then on the 6th, 11th, 16th and 21st days of the artificial cycle. All ultrasound examinations in this study were carried out by a gynecologist experienced in transvaginal ultrasonography (A.L.). Endometrial measurements were performed using a transvaginal sagittal view, from outer to outer edges at the maximal thickness point of the uterine cavity. The ovaries were screened during each examination for the purpose of monitoring any spontaneous follicular growth. Serum collections took place at 08.00, some 8 h following the last hormone administration.

Endometrial biopsy

On the 21st cycle day, following the later 10 days of combined estradiol plus progesterone administration, an endometrial biopsy was taken from all patients. The endometrial biopsies were performed immediately after a transvaginal sonography, carried out to exclude pelvic pathology and to determine the uterine position and depth. This was followed by speculum insertion and cervical cleaning with chlorhexidine gluconate 0.4% solution. The endometrial sampling was obtained with a Pipelle® (Prodimed, Neuilly-En-Thelle, France), introduced transcervically, and a single continuous sampling was performed from all aspects of the uterine body. Following fixation and staining, morphological study of the endometrial samples was based on the dating criteria of Noyes and colleagues[12]. A senior pathologist, blinded to the clinical status of the patient, performed the histological evaluation.

RESULTS

In all 29 patients who completed the study, appropriate cyclic changes in serum estradiol and progesterone levels and in endometrial thickness were observed. Serum estradiol levels on cycle days 1, 6, 11, 16 and 21 are presented in Figure 1, being significantly higher in group B on days 5 and 10. Adequate luteal phase progesterone levels were achieved with the Endometrin tablets, and no significant difference was noted in serum progesterone levels between groups on the same cycle days (Figure 2). A similar endometrial thickness was noted between groups (Figure 3). Histological evaluation of endometrial biopsies revealed adequate late-secretory endometrium, showing intraluminal secretion, tortuous glandular outline, prominent stromal edema, spiral arteries in stroma, and periarterial pseudo-decidual changes (Figure 4) in 14/15 (93.3%) patients in group A (mean day 24.2 ± 2.2) and in 13/14 (92.9%) patients in group B (mean day 23.0 ± 2.8). Only in one patient in each group (1/15 (6.6%) in group A, and 1/14 (7%) in group B) was an earlier-secretory endometrium of days 18–19 noted, although they did not differ in terms of their groups' mean hormonal and endometrial measurements. No side-effects were noted following the hormonal treatment. Spontaneous follicular growth and ovulation were sonographically ruled out in all patients.

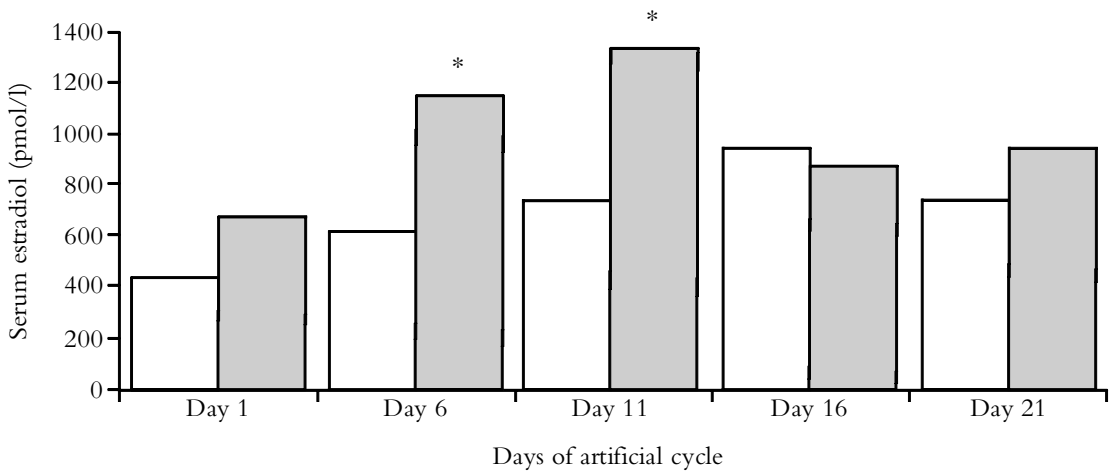


Figure 1 Mean serum estradiol levels during the artificial cycle in the 4 mg/day group (group A, □) and in the 6 mg/day group (group B, ▩). *$p < 0.05$ compared with the 4 mg/day group

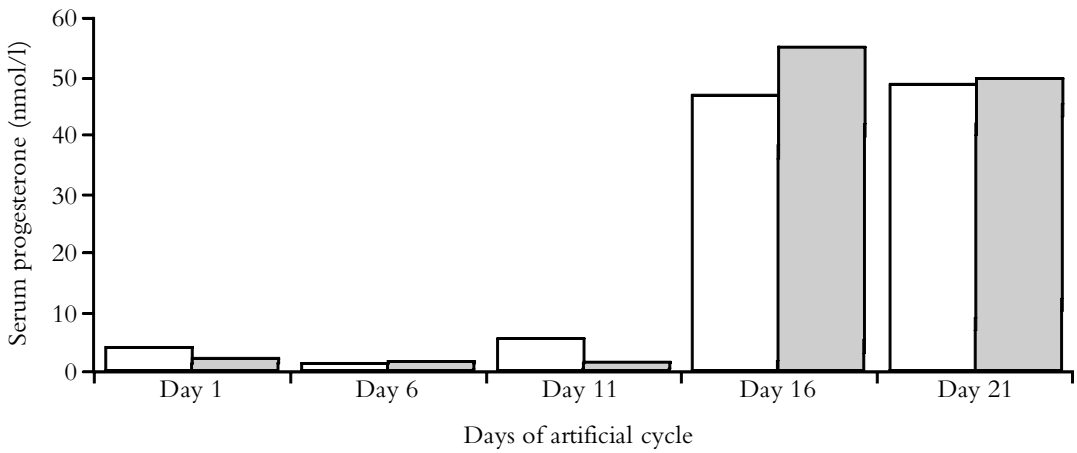


Figure 2 Mean serum progesterone levels during the artificial cycle in the 4 mg/day group (group A, □) and in the 6 mg/day group (group B, ▩)

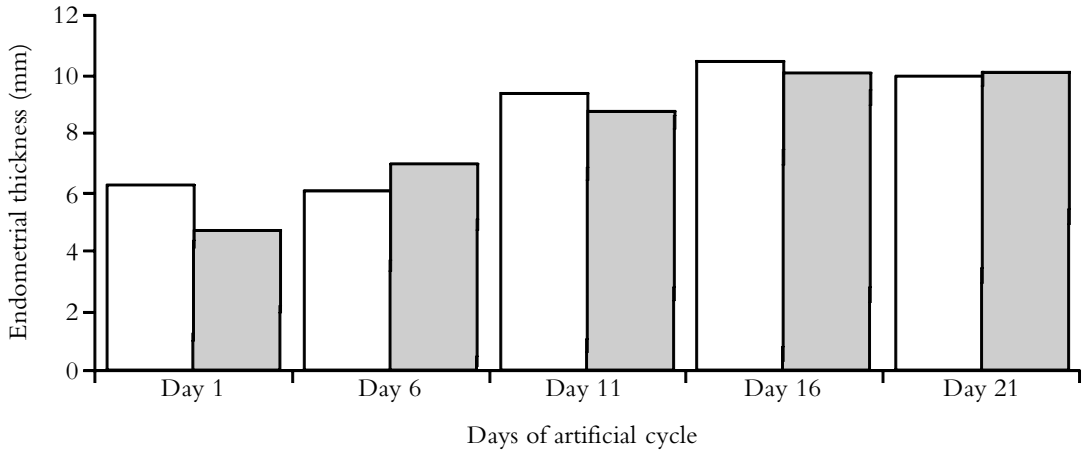


Figure 3 Mean endometrial thickness during the artificial cycle in the 4 mg/day group (group A, □) and in the 6 mg/day group (group B, ▨)

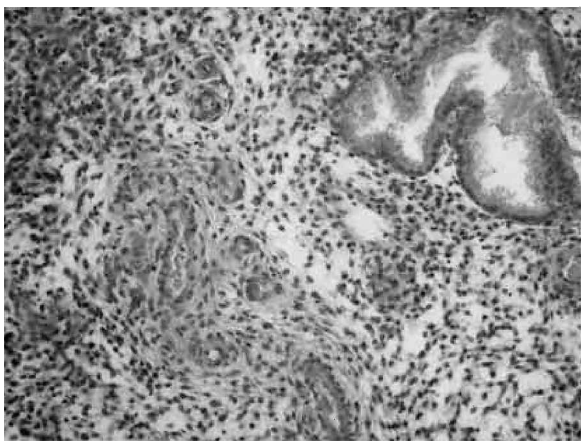


Figure 4 Late secretory endometrium (patient YR), showing intraluminal secretion, tortuous glandular outline, prominent stromal edema, spiral arteries in stroma, and periarterial pseudo-decidual changes

DISCUSSION

In this prospective randomized study, we found that a simplified sequential protocol of a fixed low dose of 4 mg/day of oral estradiol, combined sequentially with low-dose natural progesterone in vaginal tablets (Endometrin 100 mg, twice daily), allowed the attainment of adequate cyclic hormonal levels, and may induce appropriate endometrial development for embryo transfer. Adequate estradiol and progesterone priming is required for normal endometrial growth, and may be achieved with various regimens. The efficacy of three protocols of endometrial preparation using spontaneous cycles, artificial preparation and ovarian stimulation, for cryopreserved–thawed human embryo transfer (CT–ET), was studied by Tanos and colleagues[10]. No statistically significant difference was found in implantation and clinical pregnancy rates between the endometrial preparation protocols used. The authors concluded that the specific method of endometrial preparation for CT–ET had no significant impact upon the implantation rate. In fact, in most centers, artificial endometrial preparation is considered to be a convenient approach used often. Progesterone treatment for endometrial secretory transformation in artificial cycles may be administered via the oral, intramuscular or vaginal route. The oral administration of progesterone presents problems arising from its hepatic metabolism, and the resulting elevated levels of progesterone metabolites, along with too low plasma progesterone concentrations[13]. The use of intramuscular progesterone has for a long time been the gold standard of progesterone administration. It delivers higher serum progesterone levels than other routes of administration, but is associated with low patient compliance owing to marked pain at the injection site, and its prolonged use often results in gluteal abscesses[14]. Vaginal progesterone administration, although providing lower serum concentrations than intramuscular injections, was found to result, via direct local absorption, in higher tissue levels in the gynecological tract, especially the endometrium[14,15]. The vaginal route has been described as a targeted drug delivery system with uterine first-pass effect[16], and is therefore considered the best route for natural progesterone treatment. The efficacy and safety of this approach for

progesterone delivery, in luteal-phase support of fertility and IVF treatments, including artificial cycles for oocyte donation and for the transfer of frozen–thawed embryos, has previously been demonstrated[17]. Until recently, only micronized progesterone tablets at a daily dose of 900 mg (300 mg every 8 h) were available for vaginal application, described as an effective treatment for CT–ET[7]. The main drawback is the need for three daily applications of three small round tablets each time, making it very inconvenient for working patients. Gibbons and colleagues[8] compared the efficacy of a recently introduced vaginal progesterone gel with that of intramuscular progesterone within a donor-egg program. Both groups underwent Estraderm® patch–progesterone treatment in a mock cycle, finishing with an endometrial biopsy on day 26. Women with residual ovarian function received a GnRH agonist. In the intramuscular-treatment group, 100 mg progesterone was administered from cycle days 15 to 27. In the vaginal-treatment group, Crinone® 8%, a polycarbophil-based gel preparation containing 90 mg of micronized progesterone, was administered twice daily from the evening of day 14. Mean serum progesterone levels were found to be significantly lower in the Crinone group, compared with the intramuscular-progesterone group. Nevertheless, endometrial histology was 'in phase' for all subjects in both groups.

It is clear that some protocols are less convenient than others, especially with regard to the use of artificial agonadism with GnRHa for priming the artificial cycle[9]. This was recently shown to be unnecessary for endometrial preparation, as estradiol priming was sufficient to prevent a luteinizing hormone (LH) surge[11]. The above corroborates our finding that none of the studied patients showed sonographic signs of spontaneous ovulation. There are also disadvantages in the use of intramuscular progesterone[14] in multiple vaginal progesterone applications[7], and in possible allergic skin reactions described with estradiol patches[18]. With regard to progesterone gel, it must be taken into account that an important clinical parameter, the serum progesterone level, found to be significantly lower in the above Crinone-treated patients, cannot serve for monitoring a patient's response to treatment, as the endometrial appearance and thickness do not necessarily correlate with histological 'in phase' dating, as was observed in our study.

We therefore chose to compare the administration of two fixed doses of estradiol combined with a new, low-dose, vaginal natural progesterone preparation (100 mg Endometrin tablets), as these simple estradiol regimens, as well as being better handled by most patients, were previously demonstrated to allow an appropriate endometrial proliferative growth. We have previously shown, in a program of oocyte donation in patients without ovarian function, that a high pregnancy rate was achieved using the 4-mg/day dose for a particular length of artificial follicular phase[3]. The 4-mg/day fixed dose was also studied, by comparing four different estrogen doses: variable, fixed 1 mg/day, fixed 2 mg/day and fixed 4 mg/day[4]. Similar endometrial responses were shown in those treated with the fixed 4-mg dosage and those treated with the variable regimen, as previously described by our group[2].

Finally, one must consider the cost of treatment, which is expected to be lower for the oral use of a fixed low dose of 4 mg of estradiol compared with estradiol patches or even some oral variable regimens, and also lower for vaginal progesterone tablets compared with vaginal progesterone gel or multiple tablets application.

REFERENCES

1. Lutjen P, Trounson A, Leeton J, *et al.* The establishment and maintenance of pregnancy using *in vitro* fertilization and embryo donation in a patient with primary ovarian failure. *Nature (London)* 1984;307:174–5
2. Navot D, Laufer N, Kopolovic J, *et al.* Artificially induced cycles and establishment of pregnancies in the absence of ovaries. *N Engl J Med* 1986;314: 806–11
3. Younis JS, Mordel N, Lewin A. Artificial endometrial preparation for oocyte donation: the effect of estrogen stimulation on clinical outcome. *J Assist Reprod Genet* 1992;9:222–7
4. Li TC, Cooke ID, Warren MA, *et al.* Endometrial responses in artificial cycles: a prospective study

comparing four different oestrogen dosages. *Br J Obstet Gynaecol* 1992;99:751–6
5. Ben-Nun I, Siegal A, Shulman A, *et al*. Induction of artificial endometrial cycles with oestradiol implants and injectable progesterone: establishment of a viable pregnancy in a women with 17α-hydroxylase deficiency. *Hum Reprod* 1995;10: 2456–8
6. Li TC, Alpin JD, Warren MA, *et al*. Endometrial response to three different progestins in artificial cycles: a prospective, crossover study. *Fertil Steril* 1994;62:191–3
7. Simon A, Hurwitz A, Zentner B, *et al*. Transfer of frozen/thawed embryos in artificially prepared cycles with and without prior GnRH-a suppression – a prospective randomized study. *Hum Reprod* 1998;13:2712–7
8. Gibbons WE, Toner JP, Hamacher P, Kolm P. Experience with a novel vaginal progesterone preparation in a donor oocyte program. *Fertil Steril* 1998;69:96–101
9. Meldrum DR, Wisot A, Hamilton F, *et al*. Artificial agonadism and hormone replacement for oocyte donation. *Fertil Steril* 1989;52:509–11
10. Tanos V, Friedler S, Zajicek G, *et al*. The impact of endometrial preparation on implantation following cryopreserved– thawed-embryo transfer. *Gynecol Obstet Invest* 1996;4:227–31
11. Queenan JT Jr, Ramey JW, Seltman HJ, *et al*. Transfer of cryopreserved– thawed pre-embryos in a cycle using exogenous steroids without prior gonadotropin-releasing hormone agonist suppression yields favorable pregnancy results. *Hum Reprod* 1997;12:1176–80
12. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril* 1950;1:3–25
13. Nahoul K, Dehennin L, Jondet M, Roger M. Profiles of plasma estrogens, progesterone, and their metabolites after oral or vaginal administration of estradiol or progesterone. *Maturitas* 1993;16: 185–201
14. Miles RA, Paulson RJ, Lobo RA, *et al*. Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes: a comparative study. *Fertil Steril* 1994;62:485–90
15. Fanchin R, de Ziegler D, Bergeron C, *et al*. Transvaginal administration of progesterone. *Obstet Gynecol* 1997;90:396–401
16. Bulletti C, de Ziegler D, Flamigni C, *et al*. Targeted drug delivery in gynaecology: the first uterine pass effect. *Hum Reprod* 1997;12:101–6
17. Smitz J, Devroey P, Faguer B, *et al*. A prospective randomized comparison of intramuscular or intravaginal natural progesterone as a luteal phase and early pregnancy supplement. *Hum Reprod* 1992;7:168–75
18. Ross D, Whitehead M, Pike B. Development and validation of an objective method of determining skin erythema to transdermal oestradiol patches. *Br J Obstet Gynaecol* 1999;106:279–83

J Assist Reprod Genet (2008) 25:421–425
DOI 10.1007/s10815-008-9245-y

ORIGINAL ARTICLE

Thin unresponsive endometrium—a possible complication of surgical curettage compromising ART outcome

Yoel Shufaro · Alex Simon · Neri Laufer · Muhammad Fatum

Received: 31 May 2008 / Accepted: 15 August 2008 / Published online: 17 September 2008

Abstract

Purpose Endometrial thickness is important for implantation. Little data addresses the etiology of persistently thin endometrium. We present a patient cohort in order to define common features and draw conclusions.

Methods Thirteen out of 1,405 IVF patients repeatedly had thin unresponsive endometrium (<7 mm). Age, history, uterine cavity status, treatment type and outcome were examined.

Results Patient age was 35.9±5.7 years. Ten patients had a curettage performed previously. Nine patients had normal cavity and endometrium, and in four adhesions were diagnosed and removed. Out of 99 cycles performed afterwards, endometrial thickness increased in 22. ETs were performed in 49 cycles resulting in 11 pregnancies. Their outcome was eight miscarriages, two terminations due to malformations, and one live birth.

Conclusions Thin unresponsive endometrium was associated with curettage, not necessarily with intrauterine adhesions. Even if adequate thickening eventually occurred, the reproductive outcome was still very poor. Therefore other alternatives should be sought for these patients.

Keywords ART · Curettage · Thin and unresponsive endometrium

Capsule Thin unresponsive endometrium without adhesions is associated with past surgical curettage procedures and results in a very poor reproductive outcome.

Y. Shufaro (✉) · A. Simon · N. Laufer · M. Fatum
IVF Unit, The Department of Obstetrics and Gynecology,
Hadassah University Hospital, Ein Kerem,
P.O. Box 12000, Jerusalem 91120, Israel
e-mail: yoelsh@ekmd.huji.ac.il

Introduction

The importance of endometrial thickness, as measured by ultrasonographic examination, to successful *in vitro* fertilization (IVF) outcome is still under debate. While some studies have shown that this parameter is important for predicting the outcome of In Vitro Fertilization (IVF) cycles [1–8], other studies have failed to show such a positive relationship [9–14]. In a third category of studies the endometrial thickness was related to the IVF outcome, but only in correlation with other parameters [15, 16]. Different thresholds of endometrial thickness were suggested as essential for successful implantation and most demonstrated that no pregnancy was established when the thickness of the pre-ovulatory endometrium was <6 mm [17]. Nevertheless, Sundstrom [18] have reported a successful outcome of an IVF cycle in a patient with an endometrial thickness of no more than 4 mm.

This retrospective cohort study examines the clinical characteristics and background of such unique patients treated in our IVF unit, in an attempt to shed some light on the etiology and response of the endometrium to different treatment modalities.

Methods

One thousand four hundred five patients underwent IVF treatment cycles in our unit between 2004–2007. Of these we identified 13 patients who repeatedly had a thin and unresponsive endometrium during various types of treatment cycles. The ultrasonographic definition was a maximal endometrial thickness of no more than 7 mm, as measured by trans-vaginal ultrasound scans prior to ovulation or to the administration of human chorionic

gonadotropin (hCG) or progesterone, despite adequate ovarian response and serum estradiol (E_2) level. None of them had a uterine malformation or was exposed to DES in utero. All patients had a diagnostic hysteroscopy performed and intrauterine adhesions were surgically removed if found. ETs were performed on day 3 under US guidance or according to previous uterine length measurements from the same cycle. The patients' records were studied for general medical and gynecologic history, and for the different protocols which were utilized for ovulation induction and endometrial preparation. The major outcomes that were recorded from the post hysteroscopy cycles were: endometrial responsiveness to the different protocols used, serum estradiol level, the performance or cancellation of embryo transfer (ET), the number and quality of embryos transferred, clinical pregnancies and outcome.

Results

We identified 13 patients who repeatedly had thin and unresponsive endometrium, as defined by our criteria, in their past and present treatment cycles. Their characteristics are summarized in Table 1.

Four patients had tubal occlusion, four had a male factor problem, four had unexplained infertility and one patient had combined mechanical and male factor infertility. Diagnostic hysteroscopies revealed a normal appearing uterine cavity in nine women, and in four a few thin mild and scarce intra-uterine adhesions were diagnosed and removed. None of these patients had a history acute pelvic inflammatory disease (PID) or of intrauterine device (IUD) insertion. Two patients had a history of prior pelvic surgery (one patient had underwent salpingectomy and the other reconstructive tuboplasty). In one of these patients a later diagnostic laparoscopy was performed and some mild peritoneal adhesions were found. Ten out of the 13 patients had at least one surgical curettage performed in their history, and six of them had more than one. All the curettage procedures were performed in order to terminate past undesired pregnancies or following a diagnosis of missed abortion (IVF and spontaneous pregnancies). These patients were treated with different protocols of hormonal supplements in an attempt to adequately build-up and prepare the endometrium for embryo transfer. The different types of treatment were categorized into six main groups: (i) artificial cycles with exogenous estrogens administered (frozen–thawed embryos only), (ii) induced cycles with exogenous gonadotropins (fresh and frozen–thawed embryos), (iii) induced cycles with exogenous gonadotropins supplemented with exogenous estrogen, (iv) induced cycles with exogenous gonadotropins and low dose (100 mg/d) aspirin (administered during the entire treatment not just after ET), (v) induced cycles combined with sildenafil (Viagra™, Pfizer) and (vi) spontaneous cycles. In the cycles with exogenous estrogens, estradiol was administered mainly orally in incremental doses, and vaginal estradiol was added in some of the cycles. The maximal oral estradiol dose administered was 4 mg qid, and the maximal vaginal dose was 3 mg tid. Transdermal estrogen was added only in two cycles without any benefit. The ETs were performed on day 3 and were not difficult or complicated. The total number of cycles using each protocol, peak serum estradiol level, the number of cycles in which there was an adequate endometrial response, cycles with ET, the number of transferred embryos, embryo quality (according to Rijnders and Jansen [19]) and pregnancies are summarized in Table 2.

Out of 99 additional treatment cycles performed following hysteroscopy, in only 22 cycles an adequate endometrial response was achieved reaching an endometrial thickness of at least 7 mm. Eventually transfer of either of fresh or frozen thawed embryos was performed in only 49 cycles (all 13 patients had one or more ET eventually). Eleven clinical pregnancies were achieved of which eight were in the subgroup patient who had endometrial thickness of 7 mm or more. The overall pregnancy rate per transfer was 22% and much lower when calculated for the initial intent to treat cycles (11%). The pregnancy rate per ET was reasonable in the sub-group in which the endometrial response was adequate (8/22, 36.4%), and low in those who had an ET despite a thin endometrium (3/27, 11.1%). The lowest endometrial thickness in which a successful pregnancy was obtained was 6.8 mm, and this was the only pregnancy that ended in a live birth. The outcome of these pregnancies was poor; they ended in one live birth, two mid-trimester terminations due to malformations, and eight miscarriages.

Table 1 General characteristics of the 13 women who had thin and unresponsive endometrium

	Mean±SD (range)
Age	35.9±5.7 (27–42)
Gravidity	2.15±2.8 (0–11)
Parity	0.15±0.37 (0–1)
Infertility type (primary/secondary)	5/8
Infertility duration (years)	5.3 ±1.7 (3–9)
Previous IVF cycles	2.3±1 (1–4)
D&C procedures performed	1.9±2.5 (0–10)

Discussion

Endometrial receptivity is essential for successful implantation and establishment of pregnancies in both natural and

Table 2 Different treatment protocols, endometrial response, peak blood estradiol, ETs performed and number of achieved pregnancies in 13 patients presenting with thin endometrium

Treatment protocol	Cycles	Cycles with endometrial thickness ≥7 mm	Peak blood E_2 (pmol/L) mean±SD	Cycles with ET	Embryos transferred/ cycle mean±SD	Embryo score[d] mean±SD	Pregnancies (in responsive endometrium cycles)
Artificial cycle[a]	28	12	4,044±3,363	14	3.07±0.92	1.74±0.54	3 (3)
Induced cycle[b]	28	6	8,093±3,420	14	3.29±0.73	1.89±0.6	4 (3)
Induced cycle + aspirin[c]	7	3	8,577±4,218	4	3.5±0.58	1.93±0.62	1(1)
Induced cycle + estrogen[c]	18	1	8,745±4,336	9	3.3±1.0	1.97±0.61	2(1)
Induced cycle + Viagra™[c]	4	0	5,308±4,555	2	3.5±0.7	1.86±0.69	0
Spontaneous cycles[c]	14	0	642±274	6	3.17±1.17	2.0±0.58	1(0)
Total	99	22		49			11 (8)

[a] Frozen thawed embryos
[b] 22 fresh embryos+5 frozen–thawed embryos
[c] Fresh embryos
[d] According to Rijnders and Jansen [19], grade 1—no fragmentation; grade 2—<20% fragmentation; grade 3—20–50% fragmentation; grade 4> 50% fragments

ART cycles [20]. There are still no accepted criteria for evaluating endometrial receptivity in IVF patients. However, in an attempt to assess the endometrial receptivity and define uterine predictors of implantation and pregnancy, various ultrasonographic endometrial features like thickness, echogenicity and pattern were studied. Despite the existence of quite a few published studies, the prognostic value of ultrasonographic endometrial thickness measurements (and other parameters) in predicting implantation and pregnancy rates remains controversial. Some investigators have demonstrated a positive correlation between endometrial thickness and pregnancy rates [1–8, 11] while others have not found such an association [9, 10, 12–14], or only in association with other parameters [15, 16]. However, these differences may be in part attributed to different patient populations, stimulation protocols used or measurement timing. The incidence of thin endometrium in natural cycles has been reported to be 5% in women <40 years of age and 25% in 41 to 45 years old women [6]. The etiology of unresponsive endometrium and its impact on ART outcome have not been well characterized.

This retrospective observational cohort study includes 13 women who repeatedly had a thin and unresponsive endometrium in various types of treatment cycles (echogenicity and pattern data are not available). Their age was 35.9±5.7 and had they had a poor outcome of assisted reproduction treatments, despite their quite good embryo quality. One important observation which may shed some light on a possible mechanism is that ten out of 13 patients had a dilatation and curettage performed prior to the onset of their problem, and six of them had more than one curettage performed. All the curettage procedures were performed for the management of induced abortion or miscarriage. Although an association between endometrial curettage and thin unresponsive endometrium seems plausible, post curettage infertility and implantation failure was mainly attributed to the formation of intra-uterine adhesions. This small uncontrolled patient cohort does not allow us to conclude that the curettage procedures without synechiae were the cause of the injury to the endometrium. However, in this group only four out of 13 patients had intra-uterine adhesions and they were quite mild. A possible explanation to this observation might be the existence of a spectrum of post curettage endometrial injuries ranging from a thin and unresponsive (but otherwise normal) endometrium at one end, to Asherman's syndrome on the other. It is impossible to estimate the occurrence rate of such complications based on this small study. However, these post curettage sequelae are devastating to the reproductive future and should be cautiously taken into consideration when intra-uterine procedures are planned. Alternatives to the traditional dilatation and curettage procedure should be considered in women of child bearing age seeking further fertility. Pharmaceutical management of abnormal uterine bleeding, early missed and induced abortions, should be encouraged in young women. Directed hysteroscopic resection of polyps should be preferred upon undirected global curettage. Such measures might prevent the type of endometrial damage reported here.

This unfortunate group of women had undergone various empirical treatments with combinations of steroid hormones

with Silfendanil and Aspirin in an effort to improve the endometrial response as measured by its ultrasonographic thickness. These different modalities, categorized in Table 2, had various success rates in terms of adequate endometrial response (defined as an ultrasonographic thickness ≥7 mm) and pregnancy rates. The treatment plans in all cycles were tailored individually and the data was collected retrospectively. Therefore no treatment was found to be better than the other, adequate endometrial thickening was achieved in only a minority of the patients, and embryo transfers were performed in only half of the cycles. The pregnancy rate was low despite the adequate number of embryos that were transferred. The thinnest endometrium which was associated with a successful pregnancy was 6.8 mm thick, quite close to the established cutoff and in conformity to published data [17]. The outcome of these 11 pregnancies (eight miscarriages, two late terminations due to malformations and one live birth) demonstrated that these patients had a poor reproductive prognosis even if some endometrial thickening or implantation did occur. The failure of the different treatment modalities to improve the poor reproductive outcome in these patients is unfortunately in accordance to published data [20]. The high pregnancy loss rate might be the consequence of an endometrial cellular or molecular defect which is presently beyond our recognition. Further studies involving direct examination of the endometrium are required in order to establish the mechanism of such an endometrial injury. Stimulation of the endometrium by local injury using an endometrial biopsy catheter was reported to be beneficial to patients with normal endometrial measurements who had otherwise unexplained repeated implantation failures [21, 22]. However the patient population in this study differs significantly by having a functionally abnormal endometrium. The effectiveness of endometrial biopsy in patients with thin unresponsive endometrium is yet to be determined.

Once the insult occurred and the endometrium is constantly thin and unresponsive, proper counseling regarding the very low live birth rate achieved by IVF and alternatives such as surrogacy and adoption should be discussed with these patients.

References

1. Bergh C, Hillensjo T, Nilsson L. Sonographic evaluation of the endometrium in in vitro fertilization IVF cycles. A way to predict pregnancy? Acta Obstet Gynecol Scand 1992;71:624–8. doi:10.3109/00016349209006231.
2. Check JH, Nowroozi K, Choe J, Dietterich C. Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization. Fertil Steril 1991;56:1173–5.
3. Dickey RP, Olar TT, Curole DN, Taylor SN, Rye PH. Endometrial pattern and thickness associated with pregnancy outcome after assisted reproduction technologies. Hum Reprod 1992;7:418–21.
4. Gonen Y, Casper RF, Jacobson W, Blankier J. Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization. Fertil Steril 1989;52:446–50.
5. Noyes N, Liu HC, Sultan K, Schattman G, Rosenwaks Z. Endometrial thickness appears to be a significant factor in embryo implantation in in-vitro fertilization. Hum Reprod 1995;10:919–22.
6. Sher G, Herbert C, Maassarani G, Jacobs MH. Assessment of the late proliferative phase endometrium by ultrasonography in patients undergoing in-vitro fertilization and embryo transfer (IVF/ET). Hum Reprod 1991;6:232–7.
7. Zenke U, Chetkowski RJ. Transfer and uterine factors are the major recipient-related determinants of success with donor eggs. Fertil Steril 2004;82:850–6. doi:10.1016/j.fertnstert.2004.03.057.
8. Hassan HA, Saleh HA. Endometrial unresponsiveness: a novel approach to assessment and prognosis in in vitro fertilization cycles. Fertil Steril 1996;66:604–7.
9. De Geyter C, Schmitter M, De Geyter M, Nieschlag E, Holzgreve W, Schneider HP. Prospective evaluation of the ultrasound appearance of the endometrium in a cohort of 1,186 infertile women. Fertil Steril 2000;73:106–13. doi:10.1016/S0015-0282(99)00484-7.
10. Fleischer AC, Herbert CM, Sacks GA, Wentz AC, Entman SS, James AE Jr. Sonography of the endometrium during conception and nonconception cycles of in vitro fertilization and embryo transfer. Fertil Steril 1986;46:442–7.
11. Glissant A, de Mouzon J, Frydman R. Ultrasound study of the endometrium during in vitro fertilization cycles. Fertil Steril 1985;44:786–90.
12. Khalifa E, Brzyski RG, Oehninger S, Acosta AA, Muasher SJ. Sonographic appearance of the endometrium: the predictive value for the outcome of in-vitro fertilization in stimulated cycles. Hum Reprod 1992;7:677–80.
13. Oliveira JB, Baruffi RL, Mauri AL, Petersen CG, Campos MS, Franco JG Jr. Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme. Hum Reprod 1993;8:1312–5.
14. Welker BG, Gembruch U, Diedrich K, al-Hasani S, Krebs D. Transvaginal sonography of the endometrium during ovum pickup in stimulated cycles for in vitro fertilization. J Ultrasound Med 1989;8:549–53.
15. Rinaldi L, Lisi F, Floccari A, Lisi R, Pepe G, Fishel S. Endometrial thickness as a predictor of pregnancy after in-vitro fertilization but not after intracytoplasmic sperm injection. Hum Reprod 1996;11:1538–41.
16. Zhang X, Chen CH, Confino E, Barnes R, Milad M, Kazer RR. Increased endometrial thickness is associated with improved treatment outcome for selected patients undergoing in vitro fertilization–embryo transfer. Fertil Steril 2005;83:336–40. doi:10.1016/j.fertnstert.2004.09.020.
17. Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review. Hum Reprod Update 1996;2:323–35. doi:10.1093/humupd/2.4.323.
18. Sundstrom P. Establishment of a successful pregnancy following in-vitro fertilization with an endometrial thickness of no more than 4 mm. Hum Reprod 1998;13:1550–2. doi:10.1093/humrep/13.6.1550.
19. Rijnders PM, Jansen CA. The predictive value of day 3 embryo morphology regarding blastocyst formation, pregnancy and implantation rate after day 5 transfer following in-vitro fertiliza-

tion or intracytoplasmic sperm injection. Hum Reprod 1998; 13:2869–73.
20. Senturk LM, Erel CT. Thin endometrium in assisted reproductive technology. Curr Opin Obstet Gynecol 2008;20:221–8. doi:10.1097/GCO.0b013e328302143c.
21. Barash A, Dekel N, Fieldust S, Segal I, Schechtman E, Granot I. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. Fertil Steril 2003;79:1317–22. doi:10.1016/S0015-0282(03)00345-5.
22. Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R, Friedler S. Favorable influence of local injury to the endometrium in intracytoplasmic sperm injection patients with high-order implantation failure. Fertil Steril 2007;87:198–201. doi:10.1016/j.fertnstert.2006.05.062.

In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case–control study of 194 treatment cycles

Anne-Sophie Gremeau, M.D., Natasha Andreadis, M.D., Muhammad Fatum, M.D., Jo Craig, M.D., Karen Turner, M.D., Enda Mcveigh, M.D., and Tim Child, M.D.

Oxford Fertility Unit, Institute of Reproductive Sciences, Oxford, United Kingdom

Objective: To compare the outcome of unstimulated in vitro maturation (IVM) and routine IVF/intracytoplasmic sperm injection (ICSI) for women with polycystic ovaries (PCO).
Design: Retrospective case–control study.
Setting: Fertility unit.
Patient(s): Ninety-seven patients undergoing IVM were compared with 97 patients undergoing IVF. All had PCO and matched for age, infertility diagnosis, and ovulatory status.
Intervention(s): In vitro maturation cycles were unstimulated and hCG was administered 35–40 hours before oocyte retrieval. Oocytes were matured in vitro for 24–48 hours before insemination by ICSI. Endometrial priming with E_2 and P was commenced from the day of egg retrieval and one to two embryos were transferred on days 2–5 of development. Standard long protocol IVF/ICSI was used in the control group.
Main Outcome Measure(s): Live birth rate per cycle and ovarian hyperstimulation syndrome (OHSS) rate.
Result(s): Overall, 65% of IVM eggs matured in vitro in the IVM group. Implantation rates were significantly higher in the IVF group (19.4% vs. 12.9%) as clinical pregnancy rates (50.5% vs. 19.6%) and live birth rates (44.3% vs. 16.5%) than in the IVM group. The OHSS rate was significantly higher in the IVF group (8.2% vs. 0%).
Conclusion(s): In vitro maturation is a safer and simpler alternative to conventional IVF for women with PCO. It avoids difficulties of gonadotropin stimulation and the risk of OHSS but has a significantly lower live birth rate. Current research projects aim to close the success gap between IVM and IVF. (Fertil Steril® 2012;98: 355–60. ©2012 by American Society for Reproductive Medicine.)
Key Words: In vitro maturation (IVM), in vitro fertilization (IVF), polycystic ovaries (PCO), ovarian hyperstimulation syndrome (OHSS)

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/gremeauas-ivm-ivf-polycystic-ovaries-ohss/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Current standard IVF protocols use pituitary suppression with GnRH agonists or antagonists and concurrent daily injections of gonadotropins to induce multiple follicular development. Gonadotropins are expensive and their safe use requires frequent ultrasound and blood monitoring. Side effects of ovarian stimulation for IVF include abdominal bloating, breast tenderness, and mood swings. Above all, IVF is associated, particularly for women with polycystic ovaries, with an increased risk of the iatrogenic, and potentially fatal, complication of ovarian hyperstimulation syndrome (OHSS) (1, 2).

The in vitro maturation (IVM) of oocytes retrieved from unstimulated ovaries is an evolving form of fertility treatment. The aim of IVM is to simplify assisted conception along with reducing both costs and treatment complications. This is achieved through the collection of immature oocytes from unstimulated ovaries followed by their in vitro maturation for up to 48 hours and, once mature, insemination and

Received November 27, 2011; revised April 22, 2012; accepted April 27, 2012; published online May 31, 2012.
A.-S.G. has nothing to disclose. N.A. has nothing to disclose. M.F. has nothing to disclose. J.C. has nothing to disclose. K.T. has nothing to disclose. E.M. has nothing to disclose. T.C. has nothing to disclose.
Reprint requests: Anne-Sophie Gremeau, M.D., Gynecologie Obstetrique Reproduction Humaine, CHU Estaing 1 place Lucie Aubrac, 63000 Clermont Ferrand, France (E-mail: asgremeau@hotmail.com).

Fertility and Sterility® Vol. 98, No. 2, August 2012 0015-0282/$36.00

doi:10.1016/j.fertnstert.2012.04.046

fertilization. Embryo culture and transfer then occurs as per routine IVF. The first birth after IVM specifically in patients with polycystic ovaries was published by Trounson in 1994 (3). Initial IVM success rates were relatively low and only sporadic pregnancies were reported (4, 5), though more recent studies have demonstrated success rates of 21.5% (6), 22.5% (7), and 38.5% (8).

To our knowledge, no randomized controlled trials comparing IVM and IVF have been reported, and only a single case–control study from 2002 (6). That study, from McGill University, Montreal, Canada, demonstrated that for women with ovaries of polycystic morphology, the success rates achieved with IVM were approximately half of that using routine IVF. The study was undertaken 10 years ago and both IVM and IVF techniques have developed over time. The aim of this study is to compare the live birth rate of IVM and IVF treatment in the Oxford Fertility Unit for women with ultrasonographic evidence of polycystic ovaries.

MATERIALS AND METHODS

Eligible Patients

In vitro maturation and IVF treatment cycles undertaken for women with polycystic ovaries at the Oxford Fertility Unit between January 2007 and March 2010 were identified from the prospectively collated unit database. The study was a retrospective case–control analysis of routinely collected data during standard patient treatments. Consequently, institutional review board approval was not sought. Patients gave written consent, before treatment, to the use of their data for analysis. No power analysis was performed. Cases were defined as all IVM cycles performed in women with ovulatory PCO or anovulatory polycystic ovary syndrome (PCOS). An ovary was considered as polycystic (PCO) if at least 12 follicles between 2 and 9 mm were visualized on transvaginal ultrasonographic scan. Women with PCO on scan and regular ovulatory menstrual cycles were considered to have ovulatory PCO. Women with PCO on scan and menstruations 6 weeks or more apart were considered to have anovulatory PCOS. Ninety-seven women underwent 97 first IVM cycles; these were the cases. The control for each IVM cycle was an IVF cycle performed in the same period matched for female age, infertility diagnosis, and ovulatory status (see Table 1). All patients' files were manually checked to confirm the diagnosis of ovulatory PCO or PCOS and to assess for cycle cancellations, cryopreservation of all embryos, and complications such as OHSS. Women with amenorrhea received oral P tablets (Provera 5 mg three times per day during 5–7 days) to induce a withdrawal bleed.

TABLE 1

Patient characteristics in the IVF and IVM groups.

Characteristic	IVF group (n = 97)	IVM group (n = 97)	*P* value
Age	32.40 ± 3.6	32.38 ± 3.8	NS
BMI	24.19 ± 4.5	24.20 ± 4.6	NS
FSH	5.1 ± 1.8	5.5 ± 1.4	NS
Ovulatory PCO, % (n)	46.4 (45)	46.4 (45)	
Anovulatory PCOS, % (n)	53.6 (52)	53.6 (52)	
Hyperandrogenism, % (n)	16.5 (16)	20.6 (20)	NS
Etiology			
Duration	3.77 ± 2.5	4.20 ± 2.4	NS
Primary infertility	69.1 (67)	56.7 (55)	NS
Secondary infertility	30.9 (30)	43.3 (42)	NS
Mixed infertility[a]	38.1 (37)	36.1 (35)	NS
Multiple female factor infertility[b]	19.6 (19)	17.5 (17)	NS
PCO-PCOS ovulatory only	15.5 (15)	16.5 (16)	NS
PCOS anovulatory only	26.8 (26)	29.9 (29)	NS

Note: Values are presented as mean ± SD, percent, or percent (n). BMI = body mass index; NS = not significant.
[a] Mixed infertility (male and female factor).
[b] Multiple female factors include at least two of the following factors: dysovulation, tubal infertility, and uterine infertility.

Gremeau. IVM or IVF for women with PCO. Fertil Steril 2012.

In Vitro Fertilization Cycle

For IVF treatment, a long GnRH agonist protocol was used: pituitary suppression was commenced on day 21 of the menstrual cycle using intranasal nafarelin (Syneral; Pharmacia Ltd.) 400 μg twice daily. Pituitary suppression was confirmed after 3 weeks of nafarelin by a withdrawal bleed and serum $E_2 < 150$ pmol/L. Once patients were down-regulated, FSH treatment using Gonal-F (Serono Pharmaceuticals Ltd.), Puregon (Organon Laboratories Ltd.), or Menopur (Ferring Pharmaceuticals Ltd.) was started at a maximum daily SC dose of 150 IU. Response to treatment was monitored with serial ultrasonographic scans and serum E_2 levels starting from day 7 of gonadotropin stimulation. Subcutaneous hCG (Ovitrelle, Serono Pharmaceuticals Ltd.) was given as a trigger 35 hours before oocyte retrieval for which the patient was sedated with IV propofol, fentanyl, and midazolam. Conventional IVF or ICSI was used for oocyte insemination dependent on semen quality. A maximum of two embryos were replaced into the uterus trans-cervically on day 2, 3, or 5 of embryonic development. The luteal phase was supported using 400 mg daily vaginal P pessaries (Cyclogest; Shire Pharmaceuticals Ltd.) from oocyte retrieval until the pregnancy test, and then stopped regardless of the result. A urine hCG pregnancy test was performed 16 days after oocyte retrieval. Pregnancy scans were undertaken at 6 and 8 weeks' gestation.

IVM Treatment

Patients had a baseline transvaginal ultrasound scan between days 2 and 7 of menstrual bleeding, whether spontaneous or induced, to confirm the antral follicle count (AFC), ovarian accessibility for oocyte retrieval, and the absence of ovarian cysts. Gonadotropin ovarian stimulation was not used. A second scan was performed as necessary between days 6 and 10 of the cycle in ovulatory women to exclude the development of a dominant follicle (>14 mm), which is associated with poorer IVM outcome. In anovulatory women, oocyte retrieval was undertaken between days 8 and 16 of the cycle. For ovulatory PCO women, oocyte retrieval was performed before a dominant follicle of >14 mm in diameter was present. Priming with 10,000 IU hCG (Ovitrelle; Serono Pharmaceuticals Ltd.) was given SC 35 or 40 hours before oocyte retrieval. Transvaginal ultrasonography-guided oocyte collection was performed using a 17-gauge single-lumen needle (K-OSN-1735-A-60; Cook) with an aspiration pressure of 85 mmHg.

All patients received a paracervical block of 10 mL 1% lidocaine in addition to routine oocyte retrieval IV sedation. A multiple-puncture technique was used with the needle passing through the vagina into the ovary, and aspirating a number of antral follicles, before being withdrawn and flush aspirated from a test tube to prevent lumen blockage from the ovarian stromal tissue and blood. The needle was then reintroduced into the vagina and ovary until all small follicles were aspirated, without follicular flushing.

The follicular aspirates were collected in culture tubes containing Cook Flush Buffer (K-SIFB-100, Cook Europe) with 2.5 IU/mL heparin. Following their identification, oocytes were initially placed in Oocyte Washing Medium (SAGE). Oocyte maturity was assessed after the oocyte collection, as follows: oocytes were considered as immature if either a germinal vesicle was visualized in the cytoplasm or if no polar body was present (metaphase I oocyte). They were considered as mature if extrusion of the first polar body (metaphase II) had occurred. Oocyte maturation was evaluated with cumulus cells by trained embryologists on days 0, 1, and 2 after the oocyte retrieval. The oocytes are stripped just before ICSI (Fig. 1). All oocyte-handling procedures were conducted on warm stages and plates at 37°C. Mature and immature oocytes were then transferred into Oocyte Maturation Medium (SAGE) supplemented with 75 IU/L FSH and 75 IU/L LH (Menopur, Ferring) and cultured at 37°C in an atmosphere of 6% CO_2, 5% O_2, and 89% N_2 with high humidity for a maximum of 48 hours. Intracytoplasmic sperm injection (ICSI) was performed on all mature oocytes identified on either day 0 (day of oocyte collection), day 1 (24 hours after collection), or day 2 (48 hours after collection). Mature oocytes were denuded of cumulus cells, using standard procedures, prior to the ICSI procedure. Intracytoplasmic sperm injection was not performed beyond the afternoon of the second day of oocyte culture. Fertilization was assessed 16–18 hours after ICSI for the appearance of two distinct pronuclei. The embryos were cultured for 2, 3, or 5 days, depending on numbers and quality. Estradiol valerate 4 mg orally twice daily from oocyte retrieval and 400 mg daily vaginal P pessaries from the day after oocyte retrieval were used for endometrial preparation. Both drugs were continued until the pregnancy test and then on until 10 weeks' gestation if pregnant.

Results and Statistical Analysis

Mains results were biochemical pregnancy rate (bHCG positive), clinical pregnancy rate (defined by an ultrasonographic scan heart activity at 8 weeks' gestation), and live birth rate. All rates were defined per oocyte collection. We have only taken into account fresh-embryo transfer.

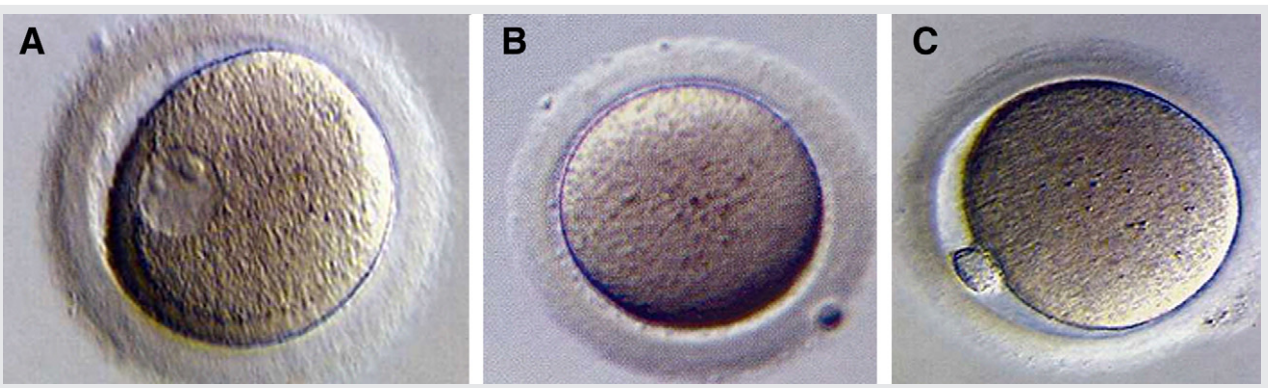
The data were analyzed using Instat3 software and the Student's *t*-test or χ^2 test as appropriate. All *P* values quoted are two sided, and values below .05 were considered statistically significant. The primary outcome measure was live birth. Secondary outcome measures included rates of oocyte maturation (number of mature oocytes obtained per number of oocytes retrieved), clinical pregnancy (fetal heart rate activity at 8 weeks' gestation), and complications (including OHSS, coasting, and freeze-all embryo cycles).

RESULTS

Ninety-seven IVM and 97 IVF cycles were identified as cases and controls, respectively. Cycles were matched for female age and ovulatory status (45 ovulatory PCO and 52 anovulatory PCOS cycles in each group) (Table 1). There were no differences in terms of age, body mass index, FSH level, and cause and duration of infertility. No dominant ovulatory cycles were found in our 97 IVM patients.

Fewer follicles were aspirated during oocyte retrieval in the IVF than IVM group (22.2 ± 9.0 vs. 35.3 ± 18.6; $P=.0005$) though the oocyte retrieval rate per aspirated follicle rate was significantly higher (75.7% vs. 48.8%; $P<.0001$) (Table 2). The oocyte IVM rate by 48 hours of culture was 65.01% in the IVM group. Oocyte maturity was assessed in all IVM cycles, but in the IVF group it was assessed only if ICSI was being used (38 cycles). The mean (±SD) number of mature oocytes was similar in the 38 IVF-ICSI cycles (12.3 ± 6.2) and 97 IVM cycles (11.2 ± 7.0; *P*=NS). Note that in the IVM group, after first examination: 68.2% of the

FIGURE 1



Different stages of oocytes maturity after denudation. (A) Germinal vesicle (immature); (B) metaphase I oocytes, absence of germinal vesicle but no polar body extrusion (immature); and (C) metaphase II oocytes, extrusion of the first polar body (mature).

Gremeau. IVM or IVF for women with PCO. Fertil Steril 2012.

TABLE 2

Cycles characteristics and outcomes of IVF versus IVM.

	IVF group (n = 97)	IVM group (n = 97)	*P* value
Cycle			
Follicles retrieved	22.2 ± 9.0	35.3 ± 18.6	<.0001
Eggs retrieved	17.2 ± 9.9	15.8 ± 7.2	NS
Oocytes/follicle	75.7	48.8	<.0001
Maturation rate	–	65.01	–
Mature oocytes obtained[a]	12.3 ± 6.2	11.2 ± 7.0	NS
Fertilization rate	61.5	62.9	NS
Cleaving embryos	9.6 ± 5.8	6.4 ± 4.8	<.0001
Embryos transferred	1.7 ± 0.6	1.9 ± 0.4	.0043
Day 2	8	13	NS
Day 3	58	80	.0008
Day 5	24	0	<.0001
No transfer	7[c]	4[d]	NS
Embryos frozen	2.6 ± 3.2	1.4 ± 2.7	.0058
Outcome			
Biochemical pregnancy	63.9 (62)	28.9 (28)	<.0001
Clinical pregnancy[b]	50.5 (49)	19.6 (19)	<.0001
Miscarriage	12.2 (6)	15.8 (3)	NS
Live birth rate	44.3 (43)	16.5 (16)	<.0001
Implantation rate	39.4	12.9	<.0001
Twins	25.6 (11)	25 (4)	NS

Note: Values are presented as mean ± SD, percent, or percent (n). NS = not significant.
[a] Oocyte maturation is not assessed on IVF, so we compared 38 ICSI cycles (metaphase II = 469) with 97 IVM cycles (metaphase II = 1,087).
[b] Clinical pregnancy = fetal heart activity at ultrasonographic scan 8 weeks' gestation.
[c] FIV = 4 freeze-all embryo for risk of OHSS + 3 failed fertilization.
[d] MIV = 3 failed fertilization + 1 freeze-all embryo for significant bleeding after oocyte retrieval.

Gremeau. IVM or IVF for women with PCO. Fertil Steril 2012.

oocytes were germinal vesicle, 28.2% were metaphase I, and only 3.6% were metaphase II. Fertilization rates were similar between the groups. More cleaving embryos were produced in the IVF group but more were transferred in the IVM group (IVF 1.7 ± 0.6 vs. IVM 1.9 ± 0.4; *P*=.0043). A maximum of two embryos were transferred per cycle in both groups. The majority of ETs were performed on day 3 after insemination in both groups, though significantly more day 5 blastocyst transfers were undertaken in the IVF compared to IVM group. In total, 11 cycles did not reach ET, 7 in the IVF group (5 freeze-all embryos because of a severe risk of OHSS, and 3 because of failed fertilization) and 4 in the IVM group (3 for failed fertilization and 1 for significant bleeding after oocyte retrieval).

The rates of live birth, pregnancy, and clinical pregnancy per cycle and the implantation rates were significantly higher in the IVF compared with the IVM group. The miscarriage and multiple pregnancy rates were similar between the groups (Table 2).

There were eight cases (8.2%) of moderate or severe OHSS requiring hospitalization in the IVF group compared to none (as expected) in the IVM group (Table 3). Among these OHSS admission cycles, five were coasted (withdrawal of gonadotropin stimulation for 1 or more days) and one had all the embryos frozen without transfer. In total, in the IVF group, nine cycles underwent coasting (i.e., four of these cycles did not end in OHSS requiring admission) and three had all their embryos frozen in order to prevent OHSS. The only complication in the IVM group was one case of needing to freeze all embryos without transfer because of excessive bleeding at

TABLE 3

Complications of IVF cycles versus IVM cycles.

	IVF group	IVM group	*P* value
OHSS moderate or severe (Golan)	8[a] (8.2)	0	.0067
Coasting	9 (9.3)	0	.0032
Freeze-all embryo	3 (3.1)	1[b] (1.0)	NS

Note: Values are presented as n (percent) or percent. NS = not significant.
[a] Eight OHSS (five were coasted and one patient had all the embryos frozen).
[b] One freeze-all embryo for massive bleeding after oocyte retrieval.

Gremeau. IVM or IVF for women with PCO. Fertil Steril 2012.

oocyte retrieval. Interestingly, we noted that 27% of the IVM cycles were performed for women who had previously undergone IVF treatment complicated by OHSS or cancelled because of risk of developing OHSS.

DISCUSSION

Our case–control study confirms that IVM is a treatment alternative to IVF for women with PCO. IVM is, however, significantly less successful than IVF. Our IVM pregnancy rates are in agreement with previous studies by Child, le Du, and Cha (6–8), with rates of 22%–27% per cycle.

Ovarian hyperstimulation syndrome is an iatrogenic condition secondary to gonadotropin ovarian stimulation. Fluid shifts from the intravascular to the third space result in volume depletion, hemoconcentration, and possible severe complications such as thromboembolism, renal impairment, respiratory distress, and rarely, death (9). Preventive strategies during IVF include withholding the hCG trigger (2), coasting (10), albumin administration at the time of oocyte retrieval (11), freezing all embryos without transfer, and in women with PCOS, cotreatment with metformin (12) or use of GnRH antagonist protocols (13). However, OHSS can occur despite the use of these strategies. The only way to absolutely avoid OHSS is to avoid ovarian stimulation, as in IVM. This is particularly true for women with risk factors for OHSS such as PCO on scan (regardless of whether ovulatory or anovulatory cycles) and younger age. Swanton et al. (1) recently reported OHSS rates in 285 first IVF cycles for younger women. Of women with anovulatory PCOS, 15.5% developed OHSS, which was statistically similar to the 12.6% rate in ovulatory PCO patients, but both were significantly higher than the 2.7% rate for women with normal ovaries. In our study, no OHSS occurred in the IVM group (as expected) compared to a rate of 8.2% in the IVF group.

Our results confirm that the IVM live birth rate is significantly lower than that for IVF for women with polycystic ovaries when controlling for patient age and other fertility factors. A previous study from 10 years ago for a similar patient group from McGill University in Montreal had similar findings (6). The live birth rate per IVM cycle was 15.9% and for IVF 26.2% though the difference did not reach statistical significance. The numbers of embryos replaced in Montreal was much higher than in the current study (in the United Kingdom, the HFEA guidance is for a maximum of two embryos replaced per cycle). In the 2002 study, the implantation rate of IVM

embryos was 9.5% compared to 12.9% in the current study and for IVF embryos 17.1% then and 39.4% now. Therefore, it appears that the success rate of IVF has improved over the past decade to a greater extent than the success rate of IVM.

The continued lower success rate of IVM could be explained by suboptimal oocyte maturation and/or impaired endometrial receptivity. An IVM oocyte retrieval is ideally performed before the development of a dominant follicle >14 mm diameter in ovulatory women (14), whereas no dominant follicle develops at all in anovulatory patients. Hence, the endometrium is exposed to lower levels of endogenous E_2 than with conventional IVF. In IVM, endometrial priming with oral estrogen is commenced from the day of oocyte collection. Consequently, the endometrium is exposed to estrogen for only a few days before ET. Russell et al. (15) reported that initiation of E_2 before IVM oocyte retrieval is associated with reduced oocyte potential and lower success rates. This unphysiological truncated E_2 endometrial priming regime currently used may explain in part the lower rate of implantation, particularly because data show that the endometrial thickness at the time of IVM ET is related to the pregnancy rate (16).

The lower implantation rate may be also due to suboptimal oocyte maturation. We can confirm nuclear maturation, with extrusion of the first polar body, but are unable to reliably assess cytoplasmic maturation, which depends on a complex cascade of events. Suboptimal and asynchronized nuclear and cytoplasmic maturation has been postulated as a cause of IVM implantation failure (17). This also explained the lower embryo quality and the lower frozen rate in IVM.

To improve IVM results, many authors had proposed a solution such as FSH priming, prolonged hCG interval, or freezing all the oocytes of embryos after IVM to proceed in a delayed transfer in a subsequent and prepared cycle (18). Regarding hCG priming, it has been reported as being of benefit in women with normal ovaries and ovulatory cycles. All of our patients had polycystic ovaries, so we did not consider such adjuvant treatment. Regarding the delay between hCH and oocyte collection, we have undertaken an internal study, after the Son paper suggested an advantage of prolonged hCG interval (19). In our unit, we did not find any difference when HCG was given 35 or 40 hours before egg collection. These data are being prepared for submission. At least, in IVM, embryo quality is poorer than IVF (suboptimal oocyte maturation). It is also known that the results of frozen-embryo transfer are poorer than after fresh cycles. For these two reasons, freeze-all during IVM is unlikely to increase the pregnancy rate, even with a better endometrial preparation.

Despite the lower success rate, IVM treatment has many advantages over standard IVF. It simplifies assisted conception treatment by using the natural cycle, with no pituitary suppression or ovarian stimulation, and no need for frequent ultrasound and blood test monitoring. During an IVM treatment cycle, patients require only one or two monitoring scans (with no blood tests), followed by oocyte retrieval and ET. This simple, unstimulated regime is well accepted by patients. However, no studies have been reported assessing the cost-effectiveness of IVM compared with IVF though the lack of need of expensive gonadotropins is a clear advantage.

More than 1300 babies have been born using IVM (20). Perinatal and obstetric outcomes of IVM pregnancies are reassuring according to the small number of studies reported. Cha et al. (21) reported similar outcomes between IVM and IVF pregnancies for women with PCOS in terms of gestational age at delivery, birthweight, and obstetric complications. No neurodevelopmental concerns during infancy and early childhood were found (22). Most studies have reported gestational age at birth and birthweight that is comparable with the general population. However, the report by Buckett et al. (23) of higher birthweights in IVM singletons compared to SC singletons highlights the need for larger studies and emphasizes the importance of future studies investigating potential epigenetic differences in IVM children.

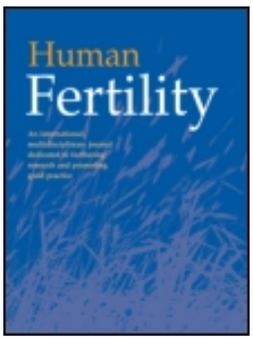
Our study only includes patients with ovaries of polycystic morphology (regardless of whether ovulatory or anovulatory cycles). The IVM success rate is related to the number of immature oocytes retrieved, which is predicted by the antral follicle count (24). Women with high antral follicle counts (i.e., PCO) therefore have a higher IVM success rate than do women with normal ovaries (25). Furthermore, the risk of OHSS during standard IVF is significantly higher in women with PCO (1), making these patients ideal candidates for IVM. The IVM success rate, for women with ovaries of polycystic morphology, is not affected by their ovulatory status (26). All IVM oocytes were fertilized using ICSI. The reason for poor fertilization rates after standard insemination has been thought to depend on altered characteristics of zona pellucida as a result of the longer culture time before insemination (26). By using ICSI, the fertilization rate of human IVM oocytes has been reported to be 70%–80% (25, 27). However, the developmental potential of the fertilized egg was similar irrespective of the insemination method. A more recent study (28) reported a higher fertilization rate with ICSI compared with IVF but higher pregnancy and implantation rates with IVF compared with ICSI. Therefore, it is not clear whether ICSI is definitely beneficial or absolutely necessary to effectively inseminate IVM oocytes in the absence of impaired sperm parameters.

We conclude that IVM is a safer and simpler, though less successful, alternative to conventional IVF for women with ovulatory PCO or PCOS. Many patients, having developed OHSS with standard IVF, are unwilling to risk the same complication again and IVM is a good treatment option. Other patients approaching assisted conception for the first time are very keen to avoid ovarian stimulation, either because of the hormonal side effects, the costs of gonadotropins, or the potential risk of OHSS. Current research projects aim to close the gap between IVM and IVF. In vitro maturation may never supersede IVF as the gold standard assisted conception treatment, but is available as an option to those requiring an alternative.

REFERENCES

1. Swanton A, Storey L, McVeigh E, Child TJ. IVF outcome in women with PCOS, PCO and normal ovarian morphology. Eur J Obstet Gynecol Reprod Biol 2010;149:68–71.
2. MacDougall MJ, Tan SL, Balen A, Jacobs HS. A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. Hum Reprod 1993;8:233–7.

3. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353–62.
4. Cha KY, Chian RC. Maturation in vitro of immature human oocytes for clinical use. Hum Reprod Update 1998;4:103–20.
5. Barnes FL, Kausche A, Tiglias J, Wood C, Wilton L, Trounson A. Production of embryos from in vitro matured primary human oocytes. Fertil Steril 1996;65: 1151–6.
6. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet Gynecol 2002;100:665–70.
7. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier MC, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod 2005;20:420–4.
8. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Hum Reprod 2000;15:165–70.
9. Golan A, Weissman A. Symposium: update on prediction and management of OHSS. A modern classification of OHSS. Reprod Biomed Online 2009;19:28–32.
10. D'Angelo A, Amso N. "Coasting" (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev 2002;(3):CD002811.
11. Aboulghar M, Evers JH, Al-Inany H. Intravenous albumin for preventing severe ovarian hyperstimulation syndrome: a Cochrane review. Hum Reprod 2002;17:3027–32.
12. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome. Hum Reprod 2006;21:1387–99.
13. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. Fertil Steril 2008;89:84–91.
14. Son WY, Chung JT, Herrero B, Dean N, Demirtas E, Holzer H, et al. Selection of the optimal day for oocyte retrieval based on the diameter of the dominant follicle in hCG-primed in vitro maturation cycles. Hum Reprod 2008; 23:2680–5.
15. Russell JB, Knezevich KM, Fabian KF, Dickson JA. Unstimulated immature oocyte retrieval: early versus midfollicular endometrial priming. Fertil Steril 1997;67:616–20.
16. Child TJ, Gulekli B, Sylvestre C, Tan SL. Ultrasonographic assessment of endometrial receptivity at embryo transfer in an in vitro maturation of oocyte program. Fertil Steril 2003;79:656–8.
17. Moor RM, Dai Y, Lee C, Fulka J Jr. Oocyte maturation and embryonic failure. Hum Reprod Update 1998;4:223–36.
18. Son WY, Chung JT, Chian RC, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Hum Reprod 2008;23:2010–6.
19. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod 1999;14: 1847–51.
20. Basatemur E, Sutcliffe A. Health of IVM children. J Assist Reprod Genet 2011;28:489–93.
21. Cha KY, Chung HM, Lee DR, Kwon H, Chung MK, Park LS, et al. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. Fertil Steril 2005;83: 1461–5.
22. Shu-Chi M, Jiann-Loung H, Yu-Hung L, Tseng-Chen S, Ming-I L, Tsu-Fuh Y. Growth and development of children conceived by in-vitro maturation of human oocytes. Early Hum Dev 2006;82:677–82.
23. Buckett WM, Chian RC, Holzer H, Dean N, Usher R, Tan SL. Obstetric outcomes and congenital abnormalities after in vitro maturation, in vitro fertilization, and intracytoplasmic sperm injection. Obstet Gynecol 2007;110: 885–91.
24. Tan SL, Child TJ, Gulekli B. In vitro maturation and fertilization of oocytes from unstimulated ovaries: predicting the number of immature oocytes retrieved by early follicular phase ultrasonography. Am J Obstet Gynecol 2002;186:684–9.
25. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertil Steril 2001;76: 936–42.
26. Nagy ZP, Cecile J, Liu J, Loccufier A, Devroey P, Van Steirteghem A. Pregnancy and birth after intracytoplasmic sperm injection of in vitro matured germinal-vesicle stage oocytes: case report. Fertil Steril 1996;65: 1047–50.
27. Mikkelsen AL. Strategies in human in-vitro maturation and their clinical outcome. Reprod Biomed Online 2005;10:593–9.
28. Söderström-Anttila V, Mäkinen S, Tuuri T, Suikkari AM. Favourable pregnancy results with insemination of in vitro matured oocytes from unstimulated patients. Hum Reprod 2005;20:1534–40.



Human Fertility

an international, multidisciplinary journal dedicated to furthering research and promoting good practice

ISSN: 1464-7273 (Print) 1742-8149 (Online) Journal homepage: https://www.tandfonline.com/loi/ihuf20

In vitro maturation and surrogacy in patients with vascular-type Ehlers–Danlos syndrome – A safe assisted reproductive technology approach

Marie-Eve Bergeron, Tim Child & Muhammad Fatum

To cite this article: Marie-Eve Bergeron, Tim Child & Muhammad Fatum (2014) In vitro maturation and surrogacy in patients with vascular-type Ehlers–Danlos syndrome – A safe assisted reproductive technology approach, Human Fertility, 17:2, 141-144, DOI: 10.3109/14647273.2014.903002

To link to this article: https://doi.org/10.3109/14647273.2014.903002

Human Fertility, 2014; 17(2): 141–144

ISSN 1464-7273 print/ISSN 1742-8149 online
DOI: 10.3109/14647273.2014.903002

informa
healthcare

ORIGINAL ARTICLE

In vitro maturation and surrogacy in patients with vascular-type Ehlers–Danlos syndrome – A safe assisted reproductive technology approach

MARIE-EVE BERGERON[1,2], TIM CHILD[1] & MUHAMMAD FATUM[1]

[1]*Nuffield Department of Obstetrics and Gynaecology, Oxford Fertility Unit, Institute of Reproductive Sciences, University of Oxford, Oxford, UK and* [2]*Department of Obstetrics and Gynaecology, Faculty of Medicine, Centre Hospitalier Universitaire de Québec, Université Laval, Québec, QC, Canada*

Abstract
Ehlers–Danlos syndrome (EDS) is an autosomal dominant connective tissue disorder with one of the highest maternal mortality rates of any condition. Patients with the vascular type of EDS are prone to spontaneous arterial and visceral ruptures. The occurrence of these severe and life-threatening complications is increased in pregnancy. Moreover, these patients carry a 50% risk of having an affected child. However, little is known about the risks of assisted conception treatments on these patients. We present the case of a 33-year-old woman suffering from EDS with a history of repeated ruptures of arterial aneurysms and a recently ruptured aneurysm of the splenic artery during her first intracytoplasmic sperm injection (ICSI) cycle who was then advised to undergo only unstimulated cycles. After a few natural ICSI cycles, the patient safely underwent two in vitro maturation cycles with pre-implantation genetic diagnosis in our unit. An unaffected blastocyst was transferred into a surrogate host. To our knowledge, this is the first case of EDS in assisted reproduction technologies including pre-implantation genetic diagnosis to be reported in the medical literature. This case has shown that unstimulated in vitro maturation and pre-implantation genetic diagnosis can safely be offered for vascular-type Ehlers–Danlos patients.

Keywords: *Oocyte maturation, preimplantation genetic diagnosis, surrogacy*

Introduction

Ehlers–Danlos syndrome (EDS) type IV, also known as the vascular type of EDS, is an uncommon autosomal dominant disorder affecting connective tissue (Pepin et al., 2000; Germain, 2007). It results from abnormalities in the synthesis and structure of collagen (Pepin et al., 2000). Affected patients are at risk of arterial and visceral ruptures, such as intestinal and uterine ruptures (Pepin et al., 2000). The diagnosis is often made only after a catastrophic complication or at post-mortem examination (Pepin et al., 2000). Women suffering from EDS type IV have an increased risk of vascular complications in pregnancy as well as a 50 per cent risk of having an affected child (Pepin et al., 2000). Moreover, it is believed that pregnancy increases the likelihood of vascular and uterine ruptures (Germain, 2007). EDS type IV has one of the highest mortality rates for pregnant women of any condition, with significant morbidity if the mother survives (Hammond & Oligbo, 2012). Many reports have been published regarding EDS and pregnancy (Pope & Nicholls, 1983; Jaleel & Olah, 2007; Kundu et al., 2006) and its catastrophic complications (Kundu et al., 2006; Combeer & Combeer, 2008; Gdynia & Huber, 2008; Björck et al., 2007; Tassart et al., 2006). The complications of EDS in pregnancy have been described in several case reports and case series, and are now better understood. However, little is known of the impact of fertility treatments on this disorder and on the reproductive strategies that are most suitable for affected patients. Furthermore, the safety of assisted reproductive technologies (ARTs) in these complex and challenging patients is poorly studied. We report a case of an EDS type IV patient who has safely undergone several IVM cycles, after experiencing a life-threatening abdominal bleed in a previous IVF cycle. The patient has given written informed consent for the publication of her medical history.

Case

A 33-year-old woman was diagnosed with EDS type IV (vascular type) 6 years previously. She suffered from a

Correspondence: Marie-Eve Bergeron, Department of Obstetrics and Gynaecology, Faculty of Medicine, Centre Hospitalier Universitaire de Québec, Université Laval, 2705 Boulevard Laurier, Bureau S-762, Québec, QC G1V 4G2, Canada. Tel: + 1-418-654-2738. E-mail: meb@mebergeron.com

(Received 23 May 2013; revised 3 November 2013; accepted 4 November 2013)

number of vascular complications including a coronary artery dissection, a right iliac artery aneurysm, a left popliteal aneurysm, and a spontaneous subcapsular liver haematoma. In order to avoid all the vascular and obstetrical complications of EDS in pregnancy as well as the genetic risk to offspring, this patient decided to undergo in vitro fertilization (IVF) plus intracytoplasmic sperm injection (ICSI) with pre-gestational diagnosis (PGD) and surrogacy. The patient went through her first fresh IVF cycle in 2010 in another unit and had a ruptured splenic artery aneurysm the day before her oocyte retrieval was planned. This life-threatening complication was successfully treated with emergency coil embolization. It was hypothesized that the supraphysiological female steroid hormonal levels (mainly oestradiol) contributed to the aneurysm rupture. For this reason, the patient was advised by her consultant vascular surgeon not to contemplate any further stimulated IVF treatment, although unstimulated ART treatment was not contraindicated.

The patient then underwent her first unstimulated natural IVF cycle, during which one oocyte was collected and fertilized normally. The embryo was screened for EDS and was found to be unaffected. The test is defined to identify the variant c.2492G > A in the COL3A1 gene which is responsible for the patient's EDS type IV. Polymerase chain reaction (PCR) was employed to amplify it to a detectable level a fragment of DNA containing the variant that is associated with the disease. The presence or absence of the variant is then determined using the minisequencing technique. Then, one microsatellite polymorphism, situated in close proximity to the defective gene, is amplified and analysed using capillary electrophoresis. The analysis of the mutation site revealed the normal copy of the COL3A1 gene. The embryo was then transferred into a surrogate host, but failed to implant. Subsequently, the patient had four unstimulated natural IVF cycles with oocyte retrievals. In total, 5 oocytes were retrieved and fertilized normally with ICSI. All embryos were vitrified on day 1 of development. On thawing, only one embryo reached the 5-cell stage on day 3 and was biopsied. However, this embryo did not continue to divide after the biopsy and the cycle was abandoned.

After these failed unstimulated IVF cycles, this patient was referred to our Unit to discuss the option of in vitro maturation (IVM) plus ICSI, PGD and surrogacy. The process of an unstimulated IVM cycle in our unit has been described recently (Gremeau et al., 2012). Having obtained the patient's informed consent, it was planned to undergo a number of unstimulated IVM cycles and to freeze all fertilized eggs on day 1 of zygote development. The plan was to achieve a satisfactory number of embryos in storage, thaw them all and culture them to day 3 for the PGD biopsy with the hope of finding some unaffected embryos to replace on day 5 into the surrogate recipient.

The patient had regular menstrual cycles lasting 26–28 days. A baseline scan was done before starting the treatment and her antral follicle count (AFC) was 11. The patient then underwent a first unstimulated IVM cycle. After ultrasound scan follow-up, the patient was given an injection of 10 000 IU of rHCG on day 6 of her natural cycle. The oocyte retrieval was performed 35 h after the trigger. On the day of the oocyte retrieval, the leading follicle measured 12 mm and there were 9 follicles. During the oocyte retrieval, extra attention was paid to the known right iliac artery aneurysm. All follicles were punctured, but only one oocyte was collected. Unfortunately, the egg was atretic and ICSI could not be performed.

One month later, the patient underwent another unstimulated IVM cycle. rHCG was prescribed on day 6 of her natural cycle with a leading follicle of 14.5 mm. The oocyte retrieval was performed 35 h later, and the leading follicle was then 16 mm with an AFC of 7. Three oocytes were collected and matured in vitro. They all fertilized normally with ICSI. According to the plan, all fertilized oocytes were frozen on day 1.

A third unstimulated IVM cycle was booked, and it was planned that all embryos would be thawed and biopsied during this cycle. However, the patient was diagnosed with a 25-mm simple ovarian cyst on day 7, and the IVM cycle had to be cancelled. However, the patient decided to go ahead with the planned frozen embryo replacement (FER) cycle into the surrogate using the embryos from previous cycles. The surrogate underwent a natural FER cycle. All three embryos were thawed and 2 embryos survived. These were both biopsied at the cleavage stage on day 3. The first embryo had 7 cells, one cell was taken for PGD, but failed to yield a diagnosis and did not continue to divide. The second embryo also had 7 cells when biopsied. One cell was taken and found to be unaffected with EDS. This unaffected embryo then progressed to the early blastocyst stage. This blastocyst was transferred on day 5 of embryo development into the surrogate host, but 7 days after the surrogate's natural LH surge. Unfortunately, the embryo did not implant.

Discussion

The vascular type of EDS is a rare connective tissue disorder caused by abnormal synthesis of type III procollagen due to COL3A1 gene mutation on chromosome 2 (Oderich et al., 2005). The resultant tissue fragility and weakness can lead to multiple complications such as visceral rupture and spontaneous arterial rupture. The mean life expectancy is 48 years, and premature death is usually caused by vascular complications (Pepin et al., 2000; Oderich et al., 2005). The inheritance is autosomal dominant: affected individuals have a 50% chance of transmitting the disease to each child. In about half of the patients, a *de novo* mutation is found (Nanayakkara et al., 2006), so they do not have any familial history of EDS.

In EDS patients, catastrophic vascular ruptures tend to occur more frequently during pregnancy and postpartum (Germain, 2007). Knowing that the mortality

rate among pregnant women affected by this disorder is estimated to be between 12% (Pepin et al., 2000; Germain, 2007; Hammond & Oligbo, 2012) and 25% (Pope & Nicholls, 1983), women are often advised to avoid pregnancy and to consider adoption (Germain, 2007). Surrogacy represents a good medical alternative to adoption in affected women who wish to have a biological child and to avoid any severe obstetrical complications. Therefore, IVF and surrogacy could be the reasonable options.

In our current patient, who previously experienced severe life-threatening vascular complications, the latest being during her first stimulated IVF cycle, and knowing that pregnancy increases the likelihood of vascular and other organ ruptures, it was agreed upon to proceed with unstimulated IVM treatment followed by PGD and embryo transfer into a surrogate host. It was clinically hypothesized that the relatively high supraphysiological levels of oestradiol, similar to the high oestrogen levels in pregnancy, might be the underlying mechanism for the increased vascular fragility especially in the locus minoris resistentiae – the thinning aneurysmatic wall. It is well known that these severe complications happen more frequently during the third trimester and during the immediate post-partum period (Germain, 2007). The increasing serum oestradiol concentration together with the associated increased vascularity throughout pregnancy might therefore explain the increased risk of vascular rupture in pregnant women, especially during the third trimester and immediate post-partum period (Germain, 2007). For these reasons, it might be advisable to avoid ovarian stimulation in these patients and to use unstimulated natural IVF or IVM treatment. Natural IVF cycles remain a good option for these patients, but sequential IVM cycles should probably be considered as an alternative, in that they may achieve more oocytes per cycle, thus decreasing the number of egg retrievals and potentially increasing the number of embryos available for PGD with the prospect of an increased success rate. Although no specific treatments can prevent complications in EDS patients, knowledge of the diagnosis can influence the reproductive counselling and prevent catastrophic complications.

PGD can be performed on cell(s) biopsied from the polar body of an oocyte or from a pre-implantation embryo with the aim of establishing a pregnancy that is unaffected by a specific genetic anomaly. In this case, biopsies were performed at the cleavage stage to avoid any risk of loss of embryos during their in vitro culture since serial IVM frozen embryos were used. PGD could have also been performed following polar body biopsy since the mutation which needed to be detected was maternal, or at the blastocyst stage. Blastocyst biopsy seems to be least disruptive to subsequent development while providing the most DNA for testing (Ly et al., 2011). However, in the absence of scientific evidence in the field of PGD on IVM oocytes and embryos, it was thought that the safest option was to biopsy all embryos at the cleavage stage and culture them to the blastocyst stage while awaiting the PGD result.

Conclusion

To our knowledge, this is the first case of EDS type IV in IVF–ICSI and IVM–ICSI with PGD and surrogacy to be reported in the literature. Many reports have been published regarding EDS in pregnancy (Hammond & Oligbo, 2012; Pope & Nicholls, 1983; Jaleel & Olah, 2007) and its catastrophic complications (Jaleel & Olah, 2007; Combeer & Combeer, 2008; Gdynia & Huber, 2008; Björck et al., 2007; Tassart et al., 2006). Clinicians' awareness and accumulated knowledge of this rare, high-risk syndrome in pregnancy have recently improved, and new obstetrical strategies are emerging to help these women (Jamard et al., 2012; Palmquist et al., 2009). In fact, new protocols for managing these patients in pregnancy could encourage more affected women to consider pregnancy. Thus, an increasing number of women may contemplate assisted reproduction techniques using PGD to eliminate the risk of transmission of their disease to their offspring. However, the risks of fertility treatments in this syndrome are unknown and, as often is the case with rare genetic disorders, unfamiliarity with the disease could compromise care (Pepin et al., 2000). In conclusion, this case has shown that IVM–ICSI and PGD can be done safely. Moreover, during this treatment, the blastocyst stage was reached, which is an important step forward for this patient. It also adds to the medical literature for PGD and gives additional reproductive choices for couples who carry the EDS mutations.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

References

Björck, M., Pigg, M., Kragsterman, B., & Bergqvist, D. (2007). Fatal bleeding following delivery: a manifestation of the vascular type of Ehlers-Danlos' syndrome. *Gynecologic and Obstetric Investigation*, 63, 173–175.

Combeer, E.L. & Combeer, A.D. (2008). A rare cause of maternal death: liver and inferior vena cava rupture due to previously undiagnosed Ehlers-Danlos syndrome type IV. *European Journal of Anaesthesiology*, 25, 765–767.

Gdynia, H.J. & Huber, R. (2008). Bilateral internal carotid artery dissections related to pregnancy and childbirth. *European Journal of Medical Research*, 13, 229–230.

Germain, D.P. (2007). Ehlers-Danlos syndrome type IV. *Orphanet Journal of Rare Diseases*, 2, 32.

Gremeau, A.S., Andreadis, N., Fatum, M., Craig, J., Turner, K., McVeigh, E., Child, T. (2012). In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. *Fertility & Sterility*, 98, 355–360.

Hammond, R. & Oligbo, N. (2012). Ehlers Danlos syndrome Type IV and pregnancy. *Archives of Gynecology and Obstetrics*, 285, 51–54.

Jaleel, S. & Olah, K. (2007). Ehlers-Danlos syndrome in pregnancy. *Journal of Obstetrics and Gynaecology,* 27, 420–421.

Jamard, A., Le Hello, C., Simonet, T., & Dreyfus, M. (2012). Vascular Ehlers-Danlos syndrome and pregnancy: an obstetrical specific

support. *Journal de Gynécologie, Obstétrique et Biologie de la Reproduction*, 41, 676–678.
Kundu, A.K., Chattopadhyay, P., Kundu, S., & Choudhury, S. (2006). Pregnancy in Ehlers-Danlos syndrome. *Journal of the Association of Physicians of India*, 54, 938.
Ly, K.D., Agarwal, A., & Nagy, Z.P. (2011). Preimplantation genetic screening: does it help or hinder IVF treatment and what is the role of the embryo? *Journal of Assisted Reproduction and Genetics,* 28, 833–849.
Nanayakkara, P.W., van Bunderen, C.C., Simsek, S., Smulders, Y.M., & Pals, G. (2006). Vascular type of Ehlers-Danlos syndrome in a patient with a ruptured aneurysm of the splenic artery. *Netherlands Journal of Medicine*, 64, 374–376.
Oderich, G.S., Panneton, J.M., Bower, T.C., Lindor, N.M., Cherry, K.J., Noel, A.A., et al. (2005). The spectrum, management and clinical outcome of Ehlers-Danlos syndrome type IV: a 30-year experience. *Journal of Vascular Surgery*, 42, 98–106.
Palmquist, M., Pappas, J.G., Petrikovsky, B., Blakemore, K., & Roshan, D. (2009). Successful pregnancy outcome in Ehlers-Danlos syndrome, vascular type. *Journal of Maternal, Fetal and Neonatal Medicine*, 22, 924–927.
Pepin, M., Schwarze, U., Superti-Furga, A., & Byers, P.H. (2000). Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. *New England Journal of Medicine*, 342, 673–680.
Pope, F.M. & Nicholls, A.C. (1983). Pregnancy and Ehlers-Danslo syndrome type IV. *Lancet*, 1, 249–250.
Tassart, S., Bernard, P., Debieve, F., Devylder, M., & Hubinont, C. (2006). Dissection of renal artery aneurysm in a pregnant woman with Elhers-Danlos disease type IV. *Journal de Gynécologie, Obstétrique et Biologie de la Reproduction*, 35, 275–279.

Original Article

Rescue *In Vitro* Maturation in Polycystic Ovarian Syndrome Patients Undergoing *In Vitro* Fertilization Treatment Who Overrespond or Underrespond to Ovarian Stimulation: Is It A Viable Option? A Case Series Study

Muhammad Fatum, M.D.[1*], Marie-Eve Bergeron, M.D.[1, 2], Caroline Ross, B.Sc.[1], Anni Ding, M.A.[1*], Ayesha Bevan, B.Sc.[1], Karen Turner, Ph.D.[1],
Tim Child, M.D.[1]

1. Oxford Fertility Unit, Institute of Reproductive Sciences, Oxford, United Kingdom
2. Department of Obstetrics and Gynaecology, Faculty of Medicine, Centre Hospitalier Universitaire de Quebéc, Université Laval, Quebéc, QC, Canada

***Corresponding Address:** Oxford Fertility Unit, Institute of Reproductive Sciences, Oxford, United Kingdom

Emails: muhammad.fatum@wrh.ox.ac.uk, annidhsg93@hotmail.com

Received: 25/June/2019, Accepted: 3/November/2019

Running title: Rescue IVM: Is It A Viable Option?

Abstract

Background: This study intends to present the role of rescue *in vitro* maturation (IVM) in polycystic ovarian syndrome (PCOS) patients undergoing *in vitro* fertilization (IVF) treatment who have inappropriate responses to ovarian stimulation.

Materials and Methods: This was a retrospective case series study of five PCOS patients undergoing IVF treatment considered for cycle cancellation due to increased risk of ovarian hyperstimulation syndrome (OHSS) as group A or poor response to ovarian stimulation as group B. Patients in group A had high oestradiol levels and recruitment of high numbers of small/intermediate sized follicles that did not meet the criteria for human chorionic gonadotropin (hCG) triggering. Patients in group B responded inadequately to hormonal stimulation despite high gonadotropin dosage. Treatment was changed to rescue IVM cycles after the patients provided consent.

Results: In group A, three IVF patients deemed to have high chances of developing OHSS as evidenced by high oestradiol levels were converted to IVM. A total of the 58/68 oocytes retrieved were mature or matured *in vitro*. There were 26 cleaving embryos obtained. Two patients had live births and one patient suffered a miscarriage. In group B, rescue IVM was implemented in two patients due to poor ovarian response (POR). A total of 22/26 oocytes retrieved were mature or matured *in vitro*. There were 13 cleaving embryos obtained. One patient had a live birth, whilst the other suffered a miscarriage.

Conclusion: Rescue IVM could be a viable option in PCOS patients undergoing IVF treatment who are unable to safely meet the criteria for hCG triggering due to overresponse to ovarian stimulation or ovarian resistance to high doses of stimulation. Conversion to IVM can still result in reasonable oocyte retrieval and lead to clinical pregnancy and live births without the risks of OHSS.

Keywords: Infertility, *In Vitro* Fertilization, *In Vitro* Maturation Techniques, Oocytes

Introduction

Ovarian superovulation with gonadotropin stimulation is still the mainstay of *in vitro* fertilization (IVF) (1). The aim of ovarian stimulation is to induce multifollicular recruitment with as much synchronized cytoplasmic and nuclear maturation as possible, and to safely obtain a higher number of mature eggs at the time of egg collection (2). Side effects of ovarian stimulation can include breast tenderness, abdominal bloating, nausea and vomiting (3). More importantly, it can lead to ovarian hyperstimulation syndrome (OHSS), particularly in women with polycystic ovarian syndrome (PCOS) (4, 5).

PCOS is probably the most frequently encountered endocrinopathy in women of reproductive age (6). It is characterized by irregular menses, hyperandrogenism, and polycystic ovaries (PCO) on ultrasound findings. The prevalence of PCOS may be as high as 15-20% (7). It is believed that harvesting more eggs would compensate for subfertility in these patients. However, ovarian responses to the same stimulation protocols may vary considerably among different PCOS patients and even among different cycles in the same patient (8).

In some cycles, patients may be overstimulated, resulting in a very high number of growing follicles and increased levels of oestradiol. This group of patients is at higher risk of developing OHSS (9-11). In addition, a large cohort of antral and preantral follicles are recruited in these overstimulated cycles, which are asynchronous and heterogeneous in their growth and development (1). Consequently, immature and mature eggs are retrieved in these cycles. In some cases, this may prove to be a complex conundrum that needs much consideration, particularly when the patient is at high risk of OHSS, as demonstrated by high hormone levels, and there is an insufficient number of large-sized follicles. In these cases, cancellation could be the only option. Coasting may not be effective or plausible, as oestradiol production may increase further (12).

On the other end of the spectrum, management of PCOS women with poor ovarian response (POR) can be an equally frustrating challenge. Despite the high number of small follicles per ovary (2-3 times that of normal) (13), there is poor follicular growth and development in response to gonadotropin stimulation. This adversely affects

mature oocyte retrieval and, more importantly, pregnancy success. Like patients at high risk of developing OHSS, these women also face the prospect of cycle cancellation.
We report a cohort of overstimulated IVF patients, as indicated by their rapidly increasing oestradiol levels and the large number of follicles, and a cohort of poor responders to ovarian stimulation who converted to rescue *in vitro* maturation (IVM) treatment. The aim of this study is to examine the rate of immature oocyte recovery and their potential for IVM from cancelled IVF cycles due to an abnormal response to gonadotropin stimulation.

Materials and Methods

Eligible patients

Unplanned IVM rescue cycles were undertaken for five PCOS patients who had abnormal responses to gonadotropin stimulation as part of their IVF treatment between 2007 and 2010 at the Oxford Fertility Clinic.

PCOS was defined according to the modified Rotterdam criteria (14) . Women who were considered to have overresponded had either high levels of oestradiol and/or a high number of growing follicles (>20 at an early stage). Conversely, women who were considered as resistant to gonadotropin stimulation either responded poorly biochemically with low oestradiol levels or had poor follicular growth as evidenced by scans. Women aged over 40 and who had more than three previous failed IVF cycles were excluded from the study. In accordance with Oxford University Ethics Committee, the study was not registered and Ethical approval was not required as data were anonymised, not identifiable by researchers and were collected before the study was formulated.

In vitro fertilization and *in vitro* maturation

Our standard protocol for IVF and IVM treatments were described previously (15).

Statistical analysis

This was a case series study produced as part of an IVM programme at Oxford Fertility Unit, UK. Statistical analysis was carried out by a biostatistician at Oxford University. Statistical analyses were done using Microsoft Excel (Microsoft Office 365). Tables were produced using Microsoft Excel (Microsoft Office 365). Graphs were produced

using GraphPad Prism 8.0.0 on Mac OSX (????, ???). The case series was reported using the case report (CASE) guidelines checklist (16).

Results

We present five cases of PCOS patients (see criteria above) aged between 31 and 39 years who each underwent an unplanned rescue IVM cycle due to an abnormal ovarian response to gonadotropin stimulation at Oxford Fertility Clinic between 2007 and 2010. They agreed to undergo immature oocyte maturation retrieval with subsequent IVM of oocytes to rescue their IVF treatment. Prior to the treatment, they all had normal ovarian reserves according to their early follicular phase follicle stimulating hormone (FSH) and antral follicle counts (AFC). The main results examined were biochemical pregnancy [beta human chorionic gonadotropin (βhCG) positive], clinical pregnancy rate (defined as heart activity at 8 weeks on an ultrasonography scan) and live birth rate. Three patients (group A) were offered the option of converting to IVM rather than cancelling their IVF cycles as they were deemed to be at risk of developing severe OHSS. Average oestradiol on the day of cancellation was 11 078 ± 5141.9 pmol/L (Fig.1). Nevertheless, none of these patients actually developed OHSS. Oocyte retrieval rate per aspirated follicle was 35%. A total of 68 oocytes were retrieved between the three patients in each group, and 58 of the 68 oocytes reached metaphase I (MI) or metaphase II (MII, Fig.2). Twenty-six cleaving embryos were obtained in group A (Table 1).

In group B, two patients were offered the option of rescue IVM cycle because they had POR to gonadotropin stimulation. Average oestradiol level of the day of cycle cancelation was 2141.5 ± 482.9 pmol/L (Fig.1). Despite their disappointing response to ovarian stimulation, 13 oocytes were retrieved from each patient. In fact, oocytes could be obtained in 33% of all follicles identified and aspirated. Eleven oocytes were mature or matured in vitro for each patient (Fig.2). A total of 13 cleaving embryos were obtained in this group.

Table 1: Baseline characteristics and outcomes for each patient. Table showing baseline characteristics of each patient, oestradiol levels on the day of cancellation of IVF treatment, as well as parameters on oocytes and embryos obtained in each case

Patients 1-3 represent group A. Patients 3-4 represent group B. Pt; Patient, no; Number, MI; Metaphase I, MII; Metaphase II, BMI; Body mass index, and IVF; *In vitro* fertilization.

Pt no.	**Age**	**BMI**	**E2 on day of cancellation**	**Oocytes retrieved**	**Oocytes reaching MI or MII (% of total)**	**No. oocytes injected**	**Fertilization rate**	**No. cleaving embryos**	**Embryos transferred**	**Pregnanc test**
1	32	23	6065	22	22 (100)	22	17 (77)	12	2	+
2	31	21	16 340	34	28 (82)	28	15 (54)	12	2	+
3	34	23	10 830	12	8 (67)	8	4 (50)	4	2	+
4	32	23	1800	13	11 (85)	11	6 (55)	5	2	+
5	39	24	2483	13	11 (85)	11	8 (73)	8	2	+

In both groups, all patients had two fresh cleavage embryos transferred on day 3 of development and all (100%) had positive pregnancy tests two weeks later. Three of the five patients (60%) gave birth to healthy singletons at term (38 and 40 weeks) or near term (35 weeks). Unfortunately, one patient in group A had a late second trimester miscarriage and one patient in group B had an early first trimester miscarriage (Fig.1). Moreover, three patients had the opportunity to store their embryos. Two patients returned for a total of three frozen embryo replacement cycles, but they were all unsuccessful.

Fig.2: Numbers of oocyte retrieved and matured. Bar chart shows the numbers of oocytes retrieved and matured for each patient. Patients 1-3 represent group A and patients 4-5 represent group B.

Discussion

Our case series study shows that rescue IVM could be a viable option in PCOS patients undergoing IVF treatment but failing to safely meet the criteria for hCG triggering because of either ovarian overresponse or underresponse to hormonal stimulation.

In our study, we did not use the conventional definition of POR as defined by the European Society of Reproduction and Embryology (ESHRE) (17). Instead, POR in our study referred specifically to PCOS patients with normal ovarian reserve and high antral follicular count (AFC), yet showed poor hormonal and follicular response despite controlled ovarian hyperstimulation (COH). It leads to reduced oocyte production, cycle cancellation and, most importantly, a reduced probability of pregnancy. It is unclear why women with PCOS can have such contrasting responses to gonadotropin stimulation, although it has been suggested that certain PCOS phenotypes may be correlated with adverse assisted reproductive outcomes (8). There is no test that can reliably predict outcome of ovarian stimulation in women with PCOS. However, anti-Müllerian hormone (AMH) on day 3 of the IVF stimulation cycle may positively predict ovarian response to gonadotropin stimulation. Oestradiol levels on the day of hCG administration and oocyte retrieval rate positively correlate with increasing AMH levels during IVF cycles in PCOS patients (18). As there is no way to reliably predict poor responders to gonadotropin stimulation, we cannot identify these women for IVM straightway. However, rescue IVM after failed IVF may provide these women with a chance of pregnancy within the same cycle of treatment.

There have been efforts to identify an algorithm based on the woman's age and markers of ovarian reserve to optimise the FSH starting dose in assisted reproductive techniques (ARTs). A recent study suggested that the application of a nomogram could lead to a more tailored approach, increasing the cost-effectiveness of infertility treatment. In general, the starting dose of FSH as calculated by the nomogram was lower than the actual prescribed dose, which might reduce the risk of OHSS. However, the authors

also suggested the inadequacy of the nomogram in PCOS patients, especially in those with high AMH levels (19). Further studies are required to assess the utility and generalisability of such nomograms. The risk of OHSS may also be reduced by the administration of adjuvant medication. Administration of D-chiro-inositol (DCI) in PCOS patients resulted in a higher ovulation rate compared to placebo (20, 21). Myo-inositol and DCI may improve many of the metabolic and hormonal dysregulations characteristic of PCOS (22), and myo-inositol seems to be able to increase oocyte quality, decrease the days of FSH stimulation before hCG administration and, hence, the risk for OHSS (23, 24).

OHSS is an iatrogenic, systemic condition secondary to gonadotropin stimulation that occurs either during the luteal phase or during pregnancy. The most common form happens a few days after the induction of follicular rupture via injection of hCG when follicular growth has been medically induced (25). Fundamentally, in OHSS, an increase in vascular permeability results in third-space fluid loss, leading to intravascular volume depletion and haemoconcentration (9). Thromboembolism is a potentially serious consequence of OHSS, and can sometimes be fatal despite treatment (26). Additionally, OHSS been reported to be linked to hepatic and renal dysfunction (27, 28), but the link between COH and renal/liver dysfunction are still debated. A study by Romito et al. (????) examined 426 patients undergoing IVF treatment and found that COH did not significantly alter renal and hepatic functions. In contrast, Giugliano et al. (29) reported a case of hepatic failure after four cycles of COH in a patient that developed severe haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. Various preventative strategies of OHSS during IVF have been suggested, such as coasting (30), co-treatment with cabergoline (31) or metformin (32), cryopreservation of embryos (33), or the administration of gonadotropin releasing hormone agonists (GnRH-agonist) instead of hCG in women treated in antagonist protocols (34). However, the only absolute way of preventing OHSS is to avoid ovarian stimulation, as in IVM. Given the evidence between COH and renal/liver dysfunction is still debated, avoiding ovarian stimulation by using IVM may have the added advantage of preventing such complications, especially when many women have already gone through multiple cycles of IVF and may be at higher inherent risk for developing renal/hepatic dysfunction.

Despite the advances in ARTs, one of the main challenges is the management of patients who have POR. To this end, luteal phase ovarian stimulation and dehydroepiandrosterone (DHEA) supplementation have shown promising results in improving outcomes in PORs. Preliminary results from a single centre pilot study by Lin et al. have demonstrated that luteal phase ovarian stimulation significantly improved oocyte retrieval and quality when compared to follicular phase ovarian stimulation in patients undergoing IVF (35). In a similar finding, Chern et al. (36), in their retrospective study, reported a potential benefit of DHEA supplementation pre-IVF cycle in PORs by showing improved oocyte retrieval rate, quality of embryos and live birth rate compared to the control group.

The success rate with IVM is associated with the number of immature oocytes obtained, which is predicted by the AFC. Women with PCOs have higher AFCs (13) and, therefore, have a comparatively increased rate of success than those with normal ovaries. Women with PCO are at significantly higher risk of developing OHSS (4, 5). In our previous study, we have reported that IVM is a simpler, safer, although less successful alternative, for women with PCO or PCOS (15). Balancing the higher success rate of IVF in PCO/PCOS women with the risk of potentially developing OHSS can be a complex dilemma. With the possibility of initial IVF treatment, and then rescue IVM if they are at significant risk of developing OHSS, we may be able to make a compromise between success rate and safety that neither IVF nor IVM alone can achieve in PCOS patients.

One of the strengths of our study is the corroboration of previous findings, not only from our own group but that of others. The concept of rescue IVM began approximately two decades ago. Coskun et al. (37) have demonstrated that immature oocytes can be recovered from cancelled human gonadotropin cycles and these oocytes can be matured *in vitro*. Later, in a related publication, Jaroudi et al. (38) reported on 18 patients who underwent IVF but were then deemed to be at significant risk of developing OHSS. These women had cycle cancellation and underwent immature oocyte retrieval with subsequent IVM. On average, 8.1 immature oocytes were retrieved from each patient and 44 embryos were transferred in 17 cycles. There were two live births; however, one baby was delivered preterm and died shortly after. The study suggested that oocytes matured *in vitro* from incomplete IVF cycles could be fertilised by intracytoplasmic

sperm injection (ICSI) and the those embryos could result in pregnancies. However, at time, the low success rate could not justify recommendation of more widespread use without further research. In our study, the average number of oocytes retrieved per patient in both groups was higher than reported by Jaroudi et al. (38). There are a number of potential explanations for this. First, the study by Jaroudi et al. (38) included not only PCOS patients, but also those with other types of infertility, such as anovulatory and unexplained cases. It is known that PCOS patients have higher numbers of follicles from which immature oocytes may be retrieved. It is also plausible that the improvements in both the IVF and IVM protocols have contributed to the higher numbers of immature oocytes picked up in our study. The live birth rate (60% overall) in our study was also higher. Again, improvements in techniques and protocols may have contributed to results; however, we are aware that our cohort is very small. In our study, the maturation rate (reaching MII) in group B (27%) was lower than that in group A (58%), which was comparable with our previous study (65%) (39). Whilst this seems to be a significant difference, it is noteworthy that the cohort size in our previous study was 94, which is considerably larger than that of our current study. It is possible that there a genuine difference exists in the ability of oocytes to mature between poor responders and overresponders, which may share the same aetiology as ovarian resistance to hormonal stimulation. The fertilization rate for both groups is similar to that reported in our previous study, which is promising as it suggests that oocytes in rescue IVM are not adversely affected by their previous exposure to gonadotropin stimulation, regardless of the ovarian response.

The main limitation of our study is the sample size - the high clinical pregnancy rate and live birth rate reported in this study are arguably influenced by the small cohort size and interpretation of these results requires caution. Whilst a biostatistician carried out the data analysis, we did not calculate the sample size required before the start of the study. This was due to logistical reasons of finding cases of cancelled IVF with subsequent agreement of undergoing IVM. Arguably this affects the generalisability of our study and the ability to draw definitive conclusions based on the findings of this mini case series. However, our aim is to highlight the possibility of IVM success in a proportion of PCOS patients who fail IVF treatment in a field that has the scope for further study and research.

IVM has an inherent advantage over conventional IVF by utilising the natural menstrual cycle, and bypassing the need for ovarian stimulation and pituitary suppression, albeit at the cost for reduced chances of success. Conventionally, IVM has been considered an alternative to IVF in women at risk of OHSS or in those who may have a POR to gonadotropin stimulation. Here, we present IVM as a potential add-on treatment, which is not considered as an alternative to IVF, but rather alongside it as a rescue strategy. The advantage is that potentially recoverable immature oocytes in cancelled cycles are not wasted and the emotional stress associated with facing a potentially cancelled cycle is reduced. Additionally, it may help prevent these patients from undergoing another costly, lengthy stimulation protocol.

Conclusion

We conclude that rescue IVM could be a viable option in PCOS patients undergoing IVF treatments who fail to safely meet the criteria for hCG triggering, either due to overresponse to ovarian stimulation or ovarian resistance to high doses of stimulation. Conversion to IVM can still result in reasonable oocyte retrieval and lead to clinical pregnancy and live births without the risks of OHSS. Further research is needed to determine the aetiology of POR and OHSS, and identify markers that will allow us to reliably predict which patients for whom IVF is less appropriate than IVM. Larger studies are needed to determine whether rescue IVM is a widely applicable strategy for women who respond inappropriately to ovarian stimulation and its success rate.

Acknowledgements

There is no financial support and conflict of interest in this study.

Authors' Contributions

M.F., C.R., T.C., K.T.; Participated in study design. M.E.B., C.R., A.B., K.T., A.D.; Performed data collection. A.D., M.E.B., M.F.; Performed data analysis and interpretation. M.E.B., A.D., M.F.; Drafted the manuscript. All authors performed

editing and finalization of the manuscript, and all authors approved the final manuscript.

References

1. Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. Hum Reprod. 2003; 18(12): 2698-2703.

2. Blockeel C, Devroey P. Optimisation of the follicular phase in IVF/ICSI. Facts Views Vis ObGyn. 2012; 4(3): 203-212.

3. Rizk B, Smitz J. Ovarian hyperstimulation syndrome after superovulation using GnRH agonists for IVF and related procedures. Hum Reprod. 1992; 7(3): 320-327.

4. MacDougall MJ, Tan SL, Balen A, Jacobs HS. A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. Hum Reprod. 1993; 8(2): 233-237.

5. Swanton A, Storey L, McVeigh E, Child T. IVF outcome in women with PCOS, PCO and normal ovarian morphology. Eur J Obstet Gynecol Reprod Biol. 2010; 149(1): 68-71.

6. Carmina E, Lobo RA. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab. 1999; 84(6): 1897-1899.

7. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol. 2013; 6: 1-13.

8. Ramezanali F, Ashrafi M, Hemat M, Arabipoor A, Jalali S, Moini A. Assisted reproductive outcomes in women with different polycystic ovary syndrome phenotypes: the predictive value of anti-Müllerian hormone. Reprod Biomed Online. 2016; 32(5): 503-512.

9. Corbett S, Shmorgun D, Claman P; Reproductive Endocrinology Infertility Committee; Special Contributor. The prevention of ovarian hyperstimulation syndrome. J Obstet Gynaecol Can. 2014; 36(11): 1024-1033.

10. Humaidan P, Nelson SM, Devroey P, Coddington CC, Schwartz LB, Gordon K, et al. Ovarian hyperstimulation syndrome: review and new classification criteria for reporting in clinical trials. Hum Reprod. 2016; 31(9): 1997-2004.

11. Smith V, Osianlis T, Vollenhoven B. Prevention of Ovarian hyperstimulation syndrome: a review. Obstet Gynecol Int. 2015; 2015: 514159.

12. Rabinovici J, Kushnir O, Shalev J, Goldenberg M, Blankstein J. Rescue of menotrophin cycles prone to develop ovarian hyperstimulation. Br J Obstet Gynaecol. 1987; 94(11): 1098-1102.

13. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-müllerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab. 2006; 91(3): 941-945.

14. Azziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. J Clin Endocrinol Metab. 2006; 91(3): 781-785.

15. Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, Mcveigh E, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. Fertil Steril. 2012; 98(2): 355-360.

16. Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D, et al. The CARE guidelines: consensus-based clinical case reporting guideline development. BMJ Case Rep. 2013; 2013. pii: bcr2013201554.

17. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of "poor response" to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011; 26(7): 1616-1624.

18. Xi W, Gong F, Lu G. Correlation of serum anti-müllerian hormone concentrations on day 3 of the in vitro fertilization stimulation cycle with assisted reproduction outcome in polycystic ovary syndrome patients. J Assist Reprod Genet. 2012; 29(5): 397-402.

19. Di Paola R, Garzon S, Giuliani S, Laganà AS, Noventa M, Parissone F, et al. Are we choosing the correct FSH starting dose during controlled ovarian stimulation for intrauterine insemination cycles? Potential application of a nomogram based on woman's age and markers of ovarian reserve. Arch Gynecol Obstet. 2018; 298(5): 1029-1035.

20. Unfer V, Orrù B, Monastra G. Inositols: from physiology to rational therapy in gynecological clinical practice. Expert Opin Drug Metab Toxicol. 2016; 12(10): 1129-1131.

21. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med. 1999; 340(17): 1314-1320.

22. Paul C, Laganà AS, Maniglio P, Triolo O, Brady DM. Inositol's and other nutraceuticals' synergistic actions counteract insulin resistance in polycystic ovarian syndrome and metabolic syndrome: state-of-the-art and future perspectives. Gynecol Endocrinol. 2016; 32(6): 431-438.

23. Laganà AS, Sapia F, La Rosa VL, Vitale SG. Comment on "Inositols: from physiology to rational therapy in gynecological clinical practice." Expert Opin Drug Metab Toxicol. 2016; 12(12): 1527.

24. Reyes-Muñoz E, Sathyapalan T, Rossetti P, Shah M, Long M, Buscema M, et al. Polycystic ovary syndrome: implication for drug metabolism on assisted reproductive techniques-a literature review. Adv Ther. 2018; 35(11): 1805-1815.

25. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update. 2002; 8(6): 559-577.

26. Cluroe AD, Synek BJ. A fatal case of ovarian hyperstimulation syndrome with cerebral infarction. Pathology. 1995; 27(4): 344-346.

27. Obrzut B, Kuczyński W, Grygoruk C, Putowski L, Kluz S, Skret A. Liver dysfunction in severe ovarian hyperstimulation syndrome. Gynecol Endocrinol. 2005; 21(1): 45-49.

28. Selter J, Wen T, Palmerola KL, Friedman AM, Williams Z, Forman EJ. Life-threatening complications among women with severe ovarian hyperstimulation syndrome. Am J Obstet Gynecol. 2019; 220(6): 575. e1-575. e11.

29. Giugliano E, Cagnazzo E, Pansini G, Vesce F, Marci R. Ovarian stimulation and liver dysfunction: Is a clinical relationship possible? A case of hepatic failure after repeated cycles of ovarian stimulation. Clin Exp Reprod Med. 2013; 40(1): 38-41.

30. Nardo LG, Cheema P, Gelbaya TA, Horne G, Fitzgerald CT, Pease EH, et al. The optimal length of "coasting protocol" in women at risk of ovarian hyperstimulation syndrome undergoing in vitro fertilization. Hum Fertil (Camb). 2006; 9(3): 175-180.

31. Kılıç N, Özdemir Ö, Başar HC, Demircan F, Ekmez F, Yücel O. Cabergoline for preventing ovarian hyperstimulation syndrome in women at risk undergoing in vitro fertilization/intracytoplasmic sperm injection treatment cycles: a randomized controlled study. Avicenna J Med. 2015; 5(4): 123-127.

32. Costello MF, Chapman M, Conway U. A systematic review and meta-analysis of randomized controlled trials on metformin co-administration during gonadotrophin ovulation induction or IVF in women with polycystic ovary syndrome. Hum Reprod Oxf Engl. 2006; 21(6): 1387-1399.

33. D'Angelo A. Ovarian hyperstimulation syndrome prevention strategies: cryopreservation of all embryos. Semin Reprod Med. 2010; 28(6): 513-518.

34. Herrero L, Pareja S, Losada C, Cobo AC, Pellicer A, Garcia-Velasco JA. Avoiding the use of human chorionic gonadotropin combined with oocyte vitrification and GnRH agonist triggering versus coasting: a new strategy to avoid ovarian hyperstimulation syndrome. Fertil Steril. 2011; 95(3): 1137-1140.

35. Lin L-T, Vitale SG, Chen S-N, Wen Z-H, Tsai H-W, Chern CU, et al. Luteal Phase Ovarian Stimulation May Improve Oocyte Retrieval and Oocyte Quality in Poor Ovarian Responders Undergoing In Vitro Fertilization: Preliminary Results from a Single-Center Prospective Pilot Study. Adv Ther. 2018 Jun 1;35(6):847–56.

36. Chern C-U, Tsui K-H, Vitale SG, Chen S-N, Wang P-H, Cianci A, et al. Dehydroepiandrosterone (DHEA) supplementation improves in vitro fertilization outcomes of poor ovarian responders, especially in women with low serum concentration of DHEA-S: a retrospective cohort study. Adv Ther. 2018; 35(6): 847-856.

37. Coskun S, Jaroudi KA, Hollanders JM, Atared AM, Roca GL. Recovery and maturation of immature oocytes in patients at risk for ovarian hyperstimulation syndrome. J Assist Reprod Genet. 1998; 15(6): 372-377.

38. Jaroudi KA, Hollanders JM, Elnour AM, Roca GL, Atared AM, Coskun S. Embryo development and pregnancies from in-vitro matured and fertilized human oocytes. Hum Reprod. 1999; 14(7): 1749-1751.

39. Gremeau A-S, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. Fertil Steril. 2012; 98(2): 355-360.

CONCLUSION: This study represents the first vibrational approach to evaluate the macromolecular changes associated with oocyte ageing. So, FPAFT-IR imaging could be considered a powerful technique to provide a biochemical fingerprint. This novel approach may represent a synergic support to evaluate oocyte quality.

Supported by: University of Ancona founding 2010 to O. Carnevali and E. Giorgini.

P-449 Wednesday, October 19, 2011

IMPAIRED DNA REPAIR AS THE ROOT CAUSE OF OOCYTE AGING. K. Oktay, E. Heytens, R. Soleimani, V. Baltaci, S. Goswami. Institute for Fertility Preservation, Obstetrics & Gynecology, New York Medical College, Valhalla, NY; Cell Biology & Anatomy, New York Medical College, New York, NY; Department of Human Genetics, Istanbul Bilim University, Istanbul, TR, Turkey; Department of Biology, Yeshiva University, New York, NY.

OBJECTIVE: The molecular mechanisms behind age-related decline in oocyte quantity and quality are unknown. Recent work indicated that women with BRCA mutations may have lower egg reserve and experience menopause earlier. Because BRCA is a key double strand DNA break (DSB) repair gene, we hypothesized that impaired DSB repair and resulting accumulation of DSBs is responsible for oocyte aging.

DESIGN: Experimental.

MATERIALS AND METHODS: Given the age related decline in oocyte yield after ovarian stimulation (41 ± 15 at 2-mo, 11 ± 5 at 9-mo), we used "young" (4-wk-old) and "old" (9-mo-old) female FVB mice (n = 24). Either primordial follicles (PDF) were assessed for DSBs by γH2AX immunostaining in ovarian sections, or GV oocytes by confocal microscopic quantification of γH2AX foci. The expression of ATM-mediated DSB repair pathway genes were analyzed by QRT-PCR in PDF captured by laser dissection (LD) as well as GV oocytes. The same was also analyzed in single human oocytes (n = 20) by QRT-PCR from young (age<27) and old (age>37) subjects.

RESULTS: γH2AX-positive PDF increased significantly in old mice compared to young (16 ± 3 vs. 10 ± 2; P=0.002). Mean number of γH2AX foci was also significantly higher in GV oocytes of old vs. young (1,279 ± 594 vs. 373 ± 258; P=0.001). By QRT-PCR from mouse GV oocytes, the expression of MRE11, a gene involved in sensing DSBs and activating repair via the ATM- pathway, was downregulated by 50-99% with age. In PDF from old mice, the expression of BRCA1 was downregulated by 77-89%. Consistent with findings in rodent oocytes, QRT-PCR of single human oocytes showed that the key genes in the DSB repair pathway was down-regulated with age.

CONCLUSION: These rodent and human data support our novel hypothesis that oocyte aging is associated with accumulation of potentially lethal and mutagenic DSBs due to the impairment of DSB repair in the aging oocyte. Active and efficient DNA repair appears to be vital for oocyte health. These findings can be paradigm-shifting in understanding oocyte aging.

Supported by: RO1 HD053112.

P-450 Wednesday, October 19, 2011

ROLE OF MELATONIN IN PREVENTING HYPOCHLOROUS ACID INDUCED ALTERATIONS IN MICROTUBULE AND CHROMOSOMAL STRUCTURE IN METAPHASE-II MOUSE OOCYTES *IN VITRO*. J. Banerjee, D. Maitra, F. Shaeib, G. M. Saed, M. P. Diamond, H. Abu-Soud. Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Wayne State University, Detroit, MI.

OBJECTIVE: Recently, we have shown that melatonin, a pineal gland hormone, can prevent hypochlorous acid (HOCl) mediated protein aggregation and hemeprotein destruction. We have demonstrated that HOCl may alter metaphase-II oocyte microtubule and chromosomal alignment. The objective of the study was to test whether melatonin prevents the impairment of oocyte quality induced by HOCl *in vitro*.

DESIGN: Dose response study.

MATERIALS AND METHODS: Metaphase–II mouse oocytes, obtained commercially, were incubated in HTF for 60 minutes. Oocytes were grouped as: control, melatonin (150, 200 nmol/mL), HOCl (10, 20, 50, 100 nmol/mL) and HOCl (50 nmol/mL) pretreated with 150 and 200 nmol/mL of melatonin. Microtubule and chromosomal alignment was studied on fixed and stained oocytes and then scored by two observers based on a previously published scoring system (*Fert Stert 1222,*Vol 88,Suppl 2, October 2007). Pearson Chi-square test, Fisher's Exact test were used compare outcomes between controls and treated groups and also amongst each group.

RESULTS: Poor scores for the spindle and chromosomes increased significantly at 50 nmol/mL of HOCl (P<0.001). Oocytes treated with melatonin only at 150 and 200 nmol/mL showed no changes; significant differences (P<0.001) were observed when oocytes exposed to 50 nmol/mL of HOCl were compared to oocytes pretreated with melatonin 200 nmol/mL.Fifty percent of the oocytes demonstrated good scores both in microtubule and chromosomal alterations when pretreated with melatonin at 150 nmol/mL compared to 0% in the only HOCl group.

CONCLUSION: HOCl alters the metaphase-II mouse oocyte spindle and chromosomal alignment in a dose dependant manner, a potential cause of poor oocyte quality in patients with endometriosis. Melatonin prevented the HOCl-mediated spindle and chromosomal damage. Melatonin supplementation could be an attractive therapeutic option to prevent oocyte damage in endometriosis or inflammatory diseases to improve fertility and reproductive outcome.

Supported by: Wayne State University.

OOCYTE MATURATION

P-451 Wednesday, October 19, 2011

IVM FOR WOMEN WITH POLYCYSTIC OVARIES? A CASE-CONTROL STUDY. T. J. Child, J. Craig, K. Turner, E. McVeigh, M. Fatum, A.-S. Gremeau. Oxford Fertility Unit, Oxford, Oxfordshire, United Kingdom.

OBJECTIVE: The in-vitro maturation (IVM) of immature oocytes retrieved from unstimulated ovaries is a promising treatment, particularly for women with polycystic ovaries (PCO). IVM is safer, simpler and cheaper than IVF. There are no published RCTs comparing IVM and IVF and only one case-control study from 2002. The aim of this study is to compare the outcome of unstimulated IVM and routine IVF/ICSI for women with ultrasonographic PCO undergoing treatment at the Oxford Fertility Unit.

DESIGN: Case-control study.

MATERIALS AND METHODS: The study included 250 women with PCO undergoing their first IVM (n = 125) or IVF (n = 125) cycle, matched for age, infertility diagnosis and ovulatory status (ovulatory PCO or anovulatory PCOS). The primary outcome measure was live birth rate per cycle. IVM cycles were unstimulated and hCG was administered 35-40h before trans-vaginal immature oocyte retrieval. Oocytes were matured in-vitro for 24-48h before insemination by ICSI. Endometrial priming with estradiol and progesterone was commenced following oocyte retrieval and 1-2 embryos transferred on day 2-5 of embryo development. Standard long protocol IVF/ICSI was used in the control group.

RESULTS: The mean age was 32.8 years in both groups. A mean (SD) of 17.4 (9.6) and 15.3 (7.4) eggs were retrieved in the IVM and IVF groups, respectively (P<0.05). 66% of IVM eggs matured in-vitro. The live birth rate per retrieval was significantly lower for IVM compared to IVF (18.4% v 42.4%; P<0.001), as were the implantation (14.2% v 37.2%; P<0.001) and clinical pregnancy rates (21.6% vs. 52.0%, P<0.001). The OHSS rate was significantly higher in the IVF group (8.8%) compared to none in the IVM group.

CONCLUSION: IVM is a safer and simpler alternative to conventional IVF for women with PCO or PCOS. IVM avoids the cost of gonadotropin stimulation and the risk of OHSS, but has a significantly lower live birth rate than IVF.

P-452 Wednesday, October 19, 2011

EFFECTS OF ANDROGEN SUBSTRATE ON BOVINE CUMULUS-OOCYTE COMPLEXES STEROIDOGENESIS DURING IN VITRO MATURATION. A. C. J. S. Rosa-e-Silva, D. L. Bulgarelli, A. A. Vireque, C. P. Pitangui, M. P. Bernuci, M. F. Silva-de-Sá. Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto - University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

OBJECTIVE: Ovarian steroids are known as important factors on the route of oocytes development. Since serum-supplemented *in vitro* maturation (IVM) media decreases estradiol secretion by bovine cumulus-oocyte complexes (COCs), the aim of this study was to assess whether this effect is caused by deficiency of substrate or by low cumulus cells aromatase activity.

DESIGN: Prospective experimental study with bovine oocytes.

Gynecol Surg (2015) 12:213–265
DOI 10.1007/s10397-015-0903-7

ABSTRACTS

Abstracts of Oral, Poster and Video Presentations British Society for Gynaecological Endoscopy Silver Jubilee Meeting 'Preparing for a Golden Era' Central Hall Westminster, London 4th & 5th June 2015

Video Presentations

FCV1- Interstitial Pregnancy: A Novel Technique Using Misoprostol, Modified Dillon's Method and Operative Laparoscopy to Minimise Blood Loss

Kuhan Dharmarajah[1*], Stewart Disu[1], Raphael Laiyemo[2]
[1]*London North West Healthcare NHS Trust, London, UK;* [2]*Sherwood Forest Hospitals NHS Foundation Trust, Nottingham, UK*

Introduction:
Interstitial pregnancy is rare with an incidence of 1:2500-5000 live births. It has a mortality rate that is 7 times higher compared to tubal pregnancies and is associated with excessive blood loss.
Case Report:
A 26 year old lady presented at 7 weeks of pregnancy with vaginal spotting. She previously had a 10 week miscarriage. A trans-vaginal ultrasound revealed a right sided interstitial pregnancy based on the Timor Tritsch et al criteria. There was a 3.5 x 4.4cm highly vascular cornual mass containing a gestation sac and yolk sac. Her β-HCG level was 85,874 IU/L. She was offered emergency laparoscopic surgery. Per rectal Misoprostol 400mcg was administered at induction of anaesthesia to increase myometrial contractility and vasoconstrict the arcuate and spiral arteries. An Endoloop was placed around the base of the pregnancy to occlude the Sampson artery. A modified Dillon's method where 20IU of Pitressin diluted with 200ml of normal saline was used to minimise cardiovascular compromise. Dillon first described dilution with 100ml of normal saline in 1958. 60mls was injected beneath the Endoloop with blanching effect. The Harmonic Ace Scalpel was used to perform a cornuostomy and right salpingectomy. Closure was in two continuous layers using Stratafix Spiral Poly Dioxanone (PDO) sutures. PerClot (hydrophilic polysaccharide) was applied for haemostasis and Hyalobarrier gel to reduce adhesion formation. The intra-operative blood loss was 300ml.
Conclusion:
This is a novel combination of modified Dillon's method with medical and surgical techniques to treat an interstitial pregnancy with minimal blood loss.

FCV2- En-bloc resection of "the butterfly area" for deep infiltrating endometriosis

Suruchi Pandey[1*], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK., Chertsey, UK*

Complete removal of deep infiltrating endometriosis is crucial to the resolution of symptoms in women suffering with severe endometriosis. We describe the technique of en-bloc resection of "the butterfly area" for deep infiltrating endometriosis. Removal of this area en-bloc ensures complete removal of the disease. Resection of "the butterfly area" includes peritoneum along with the endometriotic deposits covering bilateral pelvic sidewalls, bilateral uterosacral complexes along with the rectovaginal nodule.

Steps include:

1. Thorough pre-operative work up including transvaginal scan by a gynaecologist to diagnose recto-vaginal disease. IVP and MRI are performed if deemed necessary. Ureteric stenting is performed if extensive disease is located adjacent to ureters.
2. A pelvic survey is performed followed by adhesiolysis. Uterine and ovarian suspension is performed to optimise access.
3. Bilateral ureterolysis and subsequently resection of lateral pelvic sidewalls is performed.
4. A rectal manipulator is inserted to clearly define the rectal margins.
5. This is followed by hypogastric nerve sparing resection of deep infiltrating endometriosis and all the peritoneum over bilateral pararectal spaces.
6. Resection of peritoneum over the rectovaginal septum and resection of any nodules in this region is performed.
7. At the end of the procedure, bowel integrity test is performed using air and methylene blue.

FCV3- Fluorescent Indocyanine Sentinel Node Dissection for Endometrial Cancer

Thomas Ind[1*], Michelle Harris[1], Marielle Nobbenhuis[1]
[1]*Royal Marsden Hospital, London, UK*

We will present the first indocyanide green sentinel node dissection in the UK for endometrial cancer as part of the Royal Marsden Hospital FRIE NDS study. Flourescent Robotic Indocyanine Endoscopic Node Dissection Survey (FRIENDS).

FCV4- The 8 Steps of Excising Deep Infiltrating Endometriosis

George Goumalatsos[1*], Charlotte Smith[1], Olivier Chappatte[1]
[1]*Maidstone & Tunbridge Wells NHS Trust, Pembury, Kent, UK*

GB is a 26 year-old nulliparous woman who presented with subfertility, dysmenorrheoa, cyclical rectal bleeding, dyspareunia and intermittent pelvic pain. A first stage laparoscopy performed by a fertility specialist revealed stage IV endometriosis. She was subsequently discharged on GnRH analogues and referred to us. Pelvic MRI confirmed rectovaginal endometriosis involving the full anterior rectal wall thickness. A sigmoidoscopy revealed petechial patches in the anterior rectum suggestive of endometriosis. She understood that clearance of her endometriosis may improve her chances of conception and her pain. She was also informed that she will require bowel resection with the possibility of a colostomy. Bowel preparation was prescribed preoperatively.
Laparoscopy revealed severe endometriosis in both uterosacral ligaments, a large rectovaginal nodule eroding through the posterior fornix and a stenosing lesion in the mid rectum, affecting 2/3 of the bowel thickness. After ureteric stenting, we resected all the rectovaginal endometriosis and repaired the vagina with interrupted Monocryl sutures. The rectum was then mobilised and the colorectal team using a 33mm EEA gun, under antibiotic cover, completed an anterior resection. The ovaries were normal and the tubes patent. She had an uncomplicated recovery and was discharged home 72 hours later.
The footage of her operation has been edited using a variety of techniques, to depict the 8 basic steps of excising Deep Infiltrating Endometriosis.

FCV5- 12-week size Ectopic Pregnancy in a non-communicating uterine horn - VIDEO

Alison Montgomery[1*], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK*

Case Presentation
A 20 year old female presented to A&E with abdominal pain and chest pain. A CTPA was organised for investigation of the chest pain. The patient was subsequently found to have a positive pregnancy test, however, she consented to proceed with the investigation. This was negative for pulmonary embolus but demonstrated fluid around the spleen. HCG was 45,000 with progesterone of 41. A TV USS consequently showed a 12 week size live ectopic pregnancy with free fluid.
Laparoscopy revealed a unicornuate uterus on the left hand side with a non-communicating rudimentary horn on the right with an ectopic pregnancy within the horn and fallopian tube, attached onto the pelvic side wall. There was blood filling the pelvis. Right ureterolysis was performed and the pelvic side wall dissected to allow removal of the ectopic pregnancy and the uterine horn.
Discussion
Ectopic pregnancy is a common presentation with 1-2% of pregnancies affected. However, ectopic pregnancy within rudimentary uterine horn occurs in only 1:76,000 to 1:140,000[i]. These pregnancies have increased and there should be a high index of suspicion as the consequences are high with maternal mortality. Rapid management by laparoscopy is crucial to reduce the morbidity and mortality.
[i] Iran J Reprod Med. 2015 Jan;13(1):49-52. Unruptured rudimentary horn pregnancy presenting with acute haemoperitoneum with combined intrauterine pregnancy: A case report. Lallar M, Nandal R, Sharma D.

FCV6- Single sheet laparoscopic ventral mesh rectopexy and hysteropexy for complete uterine prolapse and full thickness rectal prolapse

Abdalla Fayyad[1*], Ivlina Pandeva[1], Shanks Gurjar[1]
[1]*Luton and Dunstable University Hospital, Luton, UK*

Aim: To evaluate the efficacy and safety of single sheet laparoscopic ventral mesh rectopexy and hysteropexy for full thickness rectal prolapse and complete uterine prolapse. A video of the procedure will be presented.
Methods: Over a 5 year period, 12 women presented with full thickness rectal prolapse and complete uterine prolapse to at least 3 cam below the introitus. The procedure involved dissection of the rectovaginal septum to the level of the pelvic floor and attaching a single sheet mesh to the ventral surface of the rectum and then to the posterior aspect of the cervix. Women were evaluated pre and post operatively with the Prolapse Quality of Life Questionnaire, and Pelvic Organ Prolapse Quantification System and Patient global Impression of improvement.
Results: All procedures were successfully completed with no complications. Complete anatomical cure of rectal prolapse was achieved in all cases and all patients reported feeling either much better or very much better on PGII. Obstructive defecation symptoms particularly incomplete evacuation persisted in 2 cases despite anatomical cure of rectal prolapse
Conclusion: Single sheet ventral mesh rectopexy can be combined with hysteropexy in women with multiple compartments prolapse of the pelvic floor.

FCV7- Ultrasonic excision of endometriosis: what you see is what you burn, or perhaps not?

Shaheen Khazali[1], Christina Stylianou[1*], Alexandros Derpapas[1]
[1]*CEMIG, London, UK*

High-frequency ultrasonic technology has less lateral thermal spread than most electrosurgery modalities. These devices, however, ultimately work by generating heat and therefore can easily cause burns to adjacent organs.
In this video, we present a case of a 42-year old woman who sustained a superficial burn to the small bowel by a Harmonic scalpel during a total laparoscopic hysterectomy. In an otherwise straightforward procedure, a small bowel burn occurred when the surgeon accidentally touched a loop of ileum with the non-active blade of the ultrasonic device, seconds after ligation of the uterine artery. A 2-3 cm longitudinal white-coloured thermal line was immediately recognised on the bowel serosa, but no further damage was revealed after careful inspection of the proximal and distal bowel. Colorectal surgeon's opinion was sought who advised conservative management without over sewing the burn line. The patient was closely observed as outpatient and was asked to attend for further blood test a few days later. She made an uneventful recovery with no sequelae.
Thermal bowel injury is the most serious type of bowel injury as it is less likely to be recognised intraoperatively and peritonitis may occur up to 14 days later. Ultrasonic devices can get very hot. The jaws of these instruments, therefore, should be kept in view at all time and the device should not be used to manipulate or grasp tissue when recently activated. Intraoperative recognition of such injuries can be life saving.

FCV8- Video case presentation of laparoscopic bilateral salpingectomy for chronic pain developed after Essure hysteroscopic sterilization showing migration of the wires via the serosa of both tubes

Claire Stewart[1*], George Botros[1]
[1]*Liverpool Women's hospital Liverpool UK*

Title: Laparoscopic Bilateral Salpingectomy for Chronic Pelvic Pain Developed After Essure Hysteroscopic Sterilization Showing Migration of the Wires Via the Serosa of Both Tubes.
A 34years old lady developed chronic pelvic pain after outpatient Essure hysteroscopic sterilization for almost a year. The hysteroscopic procedure was uneventful. Three months check by HSG confirmed wires in place and bilateral tubal blockage. Since the procedure the patient developed chronic persistent bilateral iliac fossa pain for more than 9 months. A diagnostic laparoscopy showed migration of the middle part of both wires outside the tubes. The proximal ends of the wires were seen in the uterine ostia and the distal ends of the wires in the tubal lumen. The patient agreed to proceed with removal of both tubes and the wires. The following edited video shows the above described findings and the technique of removal of the tubes and the wires by laparoscopic approach.

FCV9- Transvaginal ultrasound diagnosis and laparoscopic excision of bladder endometriosis

Tom Holland[1*], Ertan Saridogan[1], Alfred Cutner[1]
[1]*UCLH London UK*

Bladder endometriosis affects up to 4% of patients with endometriosis. The symptoms include cyclic cystitis with menstruation and occasionally cyclic haematuria. The ultrasound features of thickening of the bladder wall, adjacent and adherent to the uterus are typical and facilitate an accurate diagnosis in the majority of cases.
In this video we show a case of bladder endometriosis with a videos of the pre-operative ultrasound findings, the cystoscopy findings, bilateral insertion of ureteric Pollock catheters, laparoscopic excision of the diseased area and laparoscopic interrupted suturing of the bladder at University College London Hospital.

FCV10-The role of illuminated ureteric stents in laparoscopic excision of severe endometriosis

Ilias Nikolopoulos[1*], Graham Phillips[1]
[1]*James Cook University Hospital Middlesbrough UK*

Introduction: The incidence of ureteric injury is reported to be between 0.08% and 8% depending on the complexity of laparoscopic surgery. Laparoscopic excision of infiltrating endometriosis can be challenging and poses an increased risk for ureteric injury. We have used illuminated stents for 17 years for selected cases to prevent ureteric injury.
Objective: To describe our experience from the use of illuminated ureteric stents Rocket Uriglow^{R} during laparoscopic treatment of severe endometriosis.
Methods: Out of the 45 cases submitted to the BSGE database we identified 26 cases of treatment of severe endometriosis from January 2014 to January 2015 where Uriglow^{R} ureteric stents were used intra-operatively. We reviewed the intra-operative findings and the intra-operative and post-operative complication rates.
Results: The insertion of illuminated ureteric stents takes less than 10 min and it can reduce the need and extend of ureteric dissection and the associated morbidity. We had no ureteric injuries during this case series. All our patients experienced transient post-operative haematuria that did not affect their recovery. Two patients developed urinary tract infection at 2 and 4 weeks post-operatively (7.7%). It is difficult assess whether the infections were secondary to the insertion of the stents or the cystoscopy.
Discussion: The insertion of illuminated ureteric stents is a safe procedure that ensures easy identification of the ureters and prevents ureteric injury. It is a skill that it is easily learnt and should be considered in complex laparoscopic pelvic surgery where ureterolysis and pelvic side wall dissection proves to be difficult or risky.

Oral Presentations

FC1- Haemorrhage reduction techniques used in myomectomy surgery: a survey of UK practice

Paul Simpson[1*], Monica Mittal[2], Haitham Hamoda[2], Edward Morris[1]
[1]*Department of Obstetrics & Gynaecology Norfolk and Norwich University Hospital Norwich UK*; [2]*Department of Obstetrics & Gynaecology Kings College London UK*

Uterine myomas (fibroids) are benign tumours of the uterus but in 20-50% of women the symptoms they cause warrant treatment. Myomectomy surgery carries with it significant risks including haemorrhage, need for blood transfusion and emergency hysterectomy. These negatively impact on the aims of the surgery, patient's fertility wishes and length of hospital stay.
A Cochrane review, in 2011, reviewed 'Interventions to reduce haemorrhage during myomectomy for fibroids'. Unfortunately, this review was unable to conclude that any one approach to reducing blood loss was superior. Currently, there is significant heterogeneity in the techniques and approaches used by gynaecological surgeons.
An on-line survey was designed to collect information on surgical experience, techniques used to minimise blood loss and preferences. In particular, we gathered data on the use of Vasopressin and frequency of complications. This survey was distributed to members of the British Society of Gynaecological Endoscopy (BSGE) as a representative group of UK gynaecologists.
We will present data from 108 clinicians, which were initially presented at the RCOG World Congress in Brisbane. This will include a breakdown of the techniques used and the preferences expressed by users. In particular we will present the data related to the use of intra-operative Vasopressin-the average number of units, the use of diluent, the administration technique and the complications experienced.
Although limited by recall bias, this survey gives a good guide to UK practice. It helps to highlight the potential advrse reactions clinicians should be aware of when using Vasopressin to reduce intra-operative blood loss.

FC2- Taking it one step further to tariffs: Better Care, Better Value

Miriam O'Kane[1*], Rasiah Bharathan[1], Apryll Chase[1], Elias Kovoor[1]
[1]*Maidstone and Tunbridge Wells Hospital NHS Trust Tunbridge Wells UK*

Introduction
Hysteroscopy is the gold standard in the investigation of abnormal uterine bleeding. Outpatient hysteroscopy (OPH) is a safe and effective technique. Despite this, most hysteroscopies are still performed under general anaesthesia (GA). It is pertinent that clinicians demonstrate an understanding of healthcare rationalization. The concept of Best Practice Tariffs (BPTs), introduced by the Department of Health (DoH) in April 2010, aims to promote clinical excellence and cost effectiveness. The DoH anticipates 80% of women to receive OPH.
Methods
A prospective audit of the OPH service was carried out in order to assess current practice, success rates and overall patient satisfaction. Information on national tariffs and local costs were also sought to identify opportunities for improvement.
Results
Indication for OPH included postmenopausal bleeding, abnormal uterine bleeding and polypectomy. The overall success rate of the procedure was 98%. 23% of patients required hysteroscopy under GA. The average pain score during the procedure was 4.3 out of10 (4.2/10 for vaginoscopy, 5.3/10 with traditional hysteroscopy technique). Overall patient satisfaction score was 9.7 out of 10. A significant correlation between intra-

procedural pain and satisfaction exists (P<0.05). BPT for OPH is higher (£472) than those under GA (£268).
Conclusion
Vaginoscopic OPH is a well-tolerated procedure with minimal pain and high levels of patient satisfaction. Such positive results should encourage migration to OPH. We need to explore additional strategies for minimising discomfort. Given the financial constraints of the NHS, the leverage of financial incentives combined with clinical quality will spur an expansion of OPH service.

FC3- Development of a Core Outcome Set for Heavy Menstrual Bleeding

Natalie Cooper[1,2*], Khalid Khan[1]
[1]*Blizard Institute Queen Mary University London UK*; [2]*Royal London Hospital London UK*

Core outcome sets (COS) are an agreed, standardised set of trial outcomes that clinicians and patients consider critical or important in the management of a condition. COS are disease specific and should form the minimum data sets to be collected and reported in clinical trials of that condition. The aim of COS is to prevent selective reporting, improve data synthesis.
Heavy menstrual bleeding (HMB) is an important health issue which affects 1 in 5 women of reproductive age. Currently there is no COS for HMB. Developing a COS for HMB will ensure that future trials report useful outcomes that benefit women, clinicians and healthcare service providers alike.
COS development will follow methodology recommended by COMET (Core Outcome Measures in effectiveness Trials) and will include all relevant stakeholders. Reported outcomes are identified by literature searches, and patient workshops are held to identify outcomes that are most important to them. The outcomes are combined into a long list and a three round Delphi survey is conducted asking participants (patients and their families, clinicians, nurses) to rate the importance of each outcome to move towards consensus. A consensus meeting is held to finalise the COS. Dissemination of the HMB COS will be via publication in CROWN (Core Outcomes in Women's Health) initiative journals.
We will present results of the first stages of development of a COS for HMB and discuss the heterogeneity that exists across studies. We will also promote our HMB Delphi survey and encourage interested clinicians to register their interest.

FC4- Outpatient Endometrial Ablation: The Benefits and Advantages of Conscious Sedation

Cecilia McKee[1*], Camilla Lyon-Dean[1], David Pachter[1], Will Wight[1], Tony Chalhoub[1]
[1]*Royal Victoria Infirmary Newcastle Upon Tyne UK*

Background:
Outpatient endometrial ablation has revolutionized the management of heavy menstrual bleeding. The optimal treatment, currently advocated to be under local anaesthetic (LA) has significant limitations, which could be overcome with the use of Conscious Sedation (CS).
Aim:
To demonstrate the benefits of CS over LA for outpatient endometrial ablation, based on observational data from a large teaching hospital where Endometrial ablation under CS is standard.
Method:
LA endometrial ablation was initially introduced in this unit, but due to high patient pain scores, CS was later introduced. Data was collected prospectively (at time of procedure and before discharge) from 2007 until 2013, but retrospectively analysed. The anaesthetic protocol involves Midazolam and a variable infusion of Remifentanil
Results:
66 patients underwent Endometrial Ablation under LA (Group A), and 122 under CS (Group B). Using a Likert-type 5 point scale, (0=no pain, 1=mild pain, 2=acceptable pain, 3= very painful, 4=worst pain) 50% of patients in group B reported scores of 0, with 94% reporting acceptable pain, or less. In Group A, the majority reported scores of 3 or 4. In Group A, 14% had procedure abandoned, with no abandoned procedures in group B, and no complications related to sedation. Patient satisfaction post CS was 87%.
Conclusion:
Our data demonstrate that CS provides a safe and effective means to carry out almost all endometrial ablations in the outpatient setting. This improves patient experience, and in the longer term, with the consideration of nurse-led sedation, would prove more cost effective

FC5- Treatment decision-making and support needs in heterosexual couples living with endometriosis

Helene Mitchell[1*], Nicky Hudson[1], Lorraine Culley[1], Caroline Law[1], Elaine Denny[2], Nick Raine-Fenning[3]
[1]*De Montfort University Leicester UK*; [2]*Birmingham City University Birmingham UK*; [3]*University of Nottingham Nottingham UK*

Endometriosis impacts upon a range of domains including intimate relationships. However, previous research has focused on the woman at the expense of her partner who may also be negatively affected by the condition and its treatment.
Couples were interviewed to provide in-depth data on living with endometriosis, either as patient or partner. This paper focuses on decision-making regarding management, and couples' information and support needs.
Twenty-two heterosexual couples, together for at least 12 months, and where the woman had laparoscopically-diagnosed endometriosis, were recruited via NHS clinics, support groups and snowball sampling. Separate, in-depth, face-to-face interviews (n=44) were conducted and transcribed verbatim. Data were analysed thematically and dyadically.
Treatment decisions had implications for both partners. The majority of couples reported discussing surgical and medical options together, with men being described as 'largely supportive'. However, whilst women reflected on living with and managing the condition in the longer term, men reported a desire for a cure and, for some partners, hysterectomy was perceived as a way to "fix" endometriosis.
Healthcare professionals need to consider the role of partners in treatment decision-making and be aware that within the couple unit patients and partners may have differing views about how endometriosis should be treated.
Signposting to support groups and relevant information, along with couple-focused information that highlights the effect of endometriosis on relationships, would be welcomed by patients and their partners. In addition, men highlighted the need for advice on how best to support their partner and cope with living with endometriosis themselves.

FC6- New Laparoscopic Peritoneal Pull-Through Vaginoplasty Technique

Pravin Mhatre[1,2*], Jyoti Mhatre[2]
[1]*G S Medical college, N Wadia hospital Mumbai Maharashtra India*; [2]*Kedar hospital Mumbai Maharashtra India*

Background: Many reconstructive surgical procedures have been described for vaginal agenesis. Almost all of them are surgically challenging, multi-staged, time consuming or leave permanent scars on abdomen or skin retrieval sites.
Aim: A new simple technique using laparoscopic peritoneal pull-through in creation of neo vagina has been described.
Material Methods: Total of forty five patients with congenital absence of vagina (MRKH syndrome) were treated with laparoscopic peritoneal pull through technique between 2003 till 2014. The author has described 3

different techniques using, thin peritoneal graft, thick peritoneal graft with substratum, and combined use of peritoneum with amnion grafts (in patients with pelvic kidney peritoneum retrieval is difficult)
Results: This technique has given excellent results over a period of one to seven years of follow-up. Using the principle of Mullerionosis the peritoneum is transformed in normal multi-layer vaginal epithelium. The peritoneal lining changes to stratified squamous epithelium resembling normal vagina and having acidic Ph. Vaginal biopsies were done at various stages of follow-up, from one month to one year.
Conclusion: In conclusion the new laparoscopic peritoneal pull-through vaginoplasty offers a relatively easy surgical procedure with excellent results on long term follow up. This procedure is practically devoid of morbidity associated with other techniques. Peritoneal lining undergoes metaplasia and transforms itself in to stratified squamous epithelium resembling normal vagina. This transformation has been documented in 9 patients.
As the ovary became accessible per vaginum 3 patients underwent ovum retrieval and pregnancy using surrogate mother making this a fertility enhancing procedure.

FC7-Laparoscopic hysterectomy. Does minimal access surgery alone maximise recovery?

Joan Melendez[2,1], Zwe Magama[1], Funmilayo Odejinmi[1*]
[1]*Whipps Cross University Hospital, Barts Health NHS Trust London UK*; [2]*Royal Free London NHS Trust London UK*

Introduction
Laparoscopic surgery has been credited with multiple benefits when compared to open surgery. One of them is a more rapid return to work and normal activities. The Department for Work and Pensions is seeking to develop evidence based guidance on periods of incapacity for work following common procedures. Current recommendation for return to full activity after laparoscopic and laparoscopic assisted vaginal hysterectomy is 3 weeks. 7 weeks for abdominal hysterectomy. We wanted to find out if that is realistic.
Methods
Patients who underwent laparoscopic hysterectomy for benign condition at Whipps Cross University Hospital between 2011 and 2013 were contacted by phone. Data was collected through a questionnaire designed and validated by the authors and later analysed.
Results
33 patients were included in the study and 21 (63%) of those worked, and of those, 18 (54%) of them took 8 weeks or more to return to work. 16 (48%) described her job intensity as moderate and 4 as heavy. Overall there was no relationship to the intensity of work, the number of hours worked or job satisfaction. 12 patients did not work, 11 (33%) of them took 8 weeks or more to return to normal activities.
Conclusion
Recovery from hysterectomy can be longer than expected. Laparoscopic surgery alone without good postoperative support may not be enough. The authors feel that Enhanced Recovery programs could help maximise the benefits of laparoscopic surgery.

FC8- Validation of a new Endometriosis Surgical Scoring system (Visual Numeric Endometriosis Scoring System-VNESS) using Videotaped Laparoscopic Procedures

Abdelmonim Abdalla[1*], Shaheen Khazali[2]
[1]*Frimley Park Hospital Surrey UK;* [2]*Ashford and St Peters Hospital Chertsey UK*

Background: VNESS has been developed to facilitate clear and easy communication of intraoperative findings for endometriosis. It consists of 8 numbers, each corresponding to an area of the pelvis starting clockwise from the left adnexa, Each compartment is given a score of 0-4 depending on the severity of the disease.
Objective: This project aims to examine the inter-rater and intra-rater validity of VNESS. This is phase 2 of a bigger project. Phase 1 was development, conceptualisation and consultation, which has concluded.
Materials and methods: 63 edited videos of endometriosis laparoscopic procedures were scored by three scorers, twice using VNESS, producing 378 sets of VNESS scores. Theese were then examined for inter-rater and intra-rater agreement.
Results: VNESS showed excellent intra-rater and inter-rater agreements. The mean percentage agreement in all the 8 areas for the two rounds of scoring was between 83.9% and 87.7%. For all the scorers the mean percentage agreement in all the 8 areas for the two rounds of scoring was 85.7% (range 73.2% - 95.8%). The level of perfect agreement (the percentage of the 63 video pairs on which all scorers scored exactly the same) was strong (>90%) for adnexa, pelvic sidewall and uterovesical fold, but noticeably weaker (< 75%) for both Uterosacral Ligaments and Pouch of Douglas.
Conclusion: VNESS is a simple, intuitive and reliable system for scoring of endometriosis and may have application for audit and research. Some adjustments may be needed to optimise the system to make it more descriptive and discriminative.

FC9- Enhanced recovery pathway for laparoscopic hysterectomy, a model to follow in major gynaecological surgery. Study of a pilot protocol to assess feasibility and demonstarte measurable outcomes

Oudai Ali[1*]
[1]*West Cumberland hospital Whitehaven UK*

Introduction
Creating Enhanced recovery pathway in gynaecology will apply the best evidence to stream up practice in a very standardised steps which can be audited. The enhanced recovery will require a central investment in a team that will involve many facets of the care.
Methods
This is a study of the effectiveness of applying a pilot protocol in laparoscopic hysterectomy. In 20 cases the elements of the pathway were well applied and these included preoperative patient education and pre assessment. All cases were admitted on the same day of the operation and were given gabapentin 300mg BD and laxatives BD for 7 days postoperatively. Local anaesthetic was used at the wounds at the start of the procedure and some was left in the pelvis at the end.
Results
There was reluctance to give preoperative energy drinks in all of the 20 cases. Only 4/20 cases reported mild pain in recovery and the rest reported no pain. 2/20 were discharged on the same day and the rest were discharged on next day with no pain. 18/20 cases had no catheter on discharge from theatre and no one needed recatheterisation. 4/20 had postoperative nausea the rest were given oral fluids in recovery. There was 2/20 cases with minor complications on follow up.
Conclusion
Enhanced recovery requires leadership and change of culture on the part of surgeons, anaesthetists, and nursing staff. It is not only important to do the procedure with best of technical skills but to ensure early return to function with maximum patient satisfaction. Laparoscopic hysterectomy offers a model of care to apply to other gynaecologic procedures.

FC10- Taking the red pill or the blue pill - How to turbo charge our laparoscopic skills using neurofeedback

Zahid Khan[1*]
[1]*Birmingham Women's Hospital Birmingham West Midlands UK*

Objectives
Since the EWTD was fully implemented in 2009, it has become apparent that the experiential model of learning has to change dramatically, if the

NHS is to continue to produce safe and well-trained surgeons in this changing environment.
Attention, concentration, focus and emotional balance are key to peak performance in all areas, including laparoscopic surgery. Neurofeedback is direct quantification and training of brain function, it is brainwave biofeedback, allowing you to learn how to maintain brainwave activity associated with optimal brain function.
We present the application and promising results of brainwave training on enhanced performance of basic laparoscopic skills, consolidated by neuroplasticity.
Material
Our proposed system utilises a state-of-the-art Bluetooth EEG biosensor headset, the NeuroSky MindSet. A high-end laptop is required for data capture and data processing. Sophisticated software is used to detect the full range of brainwave activity and analyse this data using complex algorithms.
Method
6 trainee doctors with varying levels of laparoscopic experience were randomized into either receiving neurofeedback therapy prior to basic laparoscopic skills training or just receiving skills training. The test group took part in a daily session of brainwave training, for three days. The completion of simple tasks on a box trainer was timed on day four, to compare, if the application of neurofeedback sessions improved performance in the test group.
Results
The test group showed up to 12% improvement in performance of simple, directed tasks.
Conclusion
Neurofeedback therapy can play a vital role in achieving peak performance levels during laparoscopic skills training.

FC11- Virtual Reality Laparoscopic Simulator: Face Validity of Essential Gynaecological Procedures

Rasiah Bharathan[1*], James McLaren[1], Thomas Ind[1]
[1]*Department of Obstetrics and Gynaecology St.George Hospital London UK*

Introduction
Simulation-based skills training in laparoscopic surgery leads to enhanced quality of performance, reduced errors, shorter operative time and superior patient safety profile. The aim of this study was to determine trainers and trainees assessment of face validity of the Simbionix LAP MentorTM III in three essential gynaecological procedures.
Methods
27 gynaecologists (5 Consultants, 3 Senior Registrars, 13 Registrars, 6 Senior House Officers) were orientated to the training modules. Subsequently, at their convenience they performed bilateral tubal ligation, bilateral salpingo-oopherectomy and right salpingectomy, for tubal ectopic pregnancy. Following completion, a ten-point Likert-scale questionnaire was completed evaluating each task based on appearance of instruments and pelvic tissue, manoeuvring and function of instruments, response to tissue manipulation, depth perception, ergonomics of the simulator and overall utility as a training tool.
Results
The median Likert-scale scores for the appearance of instruments, hand-eye coordination and utility as a training device tasks were scored 9. The instrument manoeuvring & function of instruments, appearance of tissue and response to manipulation, depth perception, bimanual handing and simulator's ergonomics were rated a median score of 8.
Conclusion
Instrumentation, tissue depiction and response to manipulation appear to have a high face validity. The Simbionix LAP MentorTM III was regarded as a valid training tool. In our next steps, construct and predictive validity assessments will enable construction of a proficiency based curriculum. We believe the simulation based training can translate to clinical benefit in gynaecology.

FC12- Laparoscopic Myomectomy versus Open Myomectomy- A meta analysis of Randomised Controlled Trials

Thafar Al-Safar[1], Hasib Ahmed[2,1*]
[1]*Canterbury Christ Church University Medway Kent UK*; [2]*Medway Maritime Hospital Gillingham Kent UK*

Background: Leiomyomata are the commonest benign tumours of the female genital tract and are associated with a number of symptoms including difficulty to conceive. Leiomyomata can be surgically removed with preservation of the uterus in women with symtomatic fibroids who wish to retain their fertility. The procedure, myomectomy can be achieved via laparotomy, laparoscopically or hysteroscopically depending on the site, size and type of myoma. There is paucity of data with regards to which approach is associated with the best outcomes in terms of subsequent pregnancies.
Method: A systematic review to assess pregnancy rates after laparoscopic myomectomy compared with pregnancy rates follwing open myomectomy is presented. The following data bases were searched: PubMed Central, Medline, BioMed Central, CINAHL (EBSCO), ScienceDirect, Cochrane library ,Google search in general and Google scholarly. Studies which met the inclusion criteria were selected and analysed.
Results: Evidence from and Meta-analysis of the two randomised control trials which met the review criteria show no significant difference between laparoscopic and open myomectomy for large myomas with regards to subsequent fecundity, in women from the reproductive age group. The laparoscopic approach, if it is practicable, is associated with a number of patient advantages including less post operative pain, less fever, reduced blood loss, shorter length of hospital stay and faster return to normal activity.
Conclusions: The surgical approach to myomectomy does not appear to influence the subsequent pregnancy rate or outcomes. Interpretation is guarded because of the small number of studies eligible for analysis. Further large studies are required to validate the findings. The laparoscopic approach is, however, associated with a number of patient advantages including faster recovery and should be the method of choice.

FC13- Introduction of a novel approach to maintaining Pneumoperitoneum at Total Laparoscopic Hysterectomy (TLH)

Lorna Hutchinson[1], Sirkhar Sircar[1], David McMurray[1], Karina Datsun[1*], Mohammed Allam[1]
[1]*NHS Lanarkshire Wishaw UK*

During TLH diathermy is used to circumferentially cut around the uterine manipulator. At this stage in the procedure CO2 can be lost.
We have developed a fluid filled "donut" which can be placed inside the vagina. This maintains pneumoperitoneum at the time of removing the uterus from the vagina. Once the specimen has been removed the "donut" can then seal the lower vagina while suturing takes place in order to avoid ongoing loss of CO2.

The following equipment is required:

- condom
- silastic foley catheter 14
- 60ml syringe
- suture material for tying (silk)
- Sterile water

1. The catheter is inserted inside the condom (3-4 cm of the catheter tip should be placed inside the condom)
2. Silk is used to tie a knot about 1cm from the end of the condom so it is fixed to the catheter

3. The tip of the condom should be then be placed alongside the above knot.
4. The tip of the condom is tied in place using the suture material forming a donut
5. The integrity of the donut is checked by filling it with 60ml of sterile water via the catheter.
6. Following insertion of the uterine manipulator the donut is threaded over the handle of the manipulator and pushed up the vagina close to the cup.
7. Prior to excising the cervix the donut is inflated with sterile water.
8. Following removal of the specimen the donut is replaced in the lower vagina to ensure ongoing pneumoperitoneum.

FC14- Opportunistic Laparoscopic Salpingectomy (OLS): An Opportunity for Training

Rasiah Bharathan[1*], Jody Taylor[1], James McLaren[2] Iranthi Meththananda[3], Rachel Read[4], Mallika Azizia[3], Sujeewa Fernando[4], Shreelatha Datta[3], Elias Kovoor[1]
[1]Maidstone and Tunbridge Wells Hospitals NHS Trust Tunbridge Wells UK; [2]Croydon University Hospital NHS Trust Croydon UK; [3]Kings College Hospital NHS Trust London UK; [4]Wrexham Maelor Hospital Wales UK

Introduction
OLS offers potential benefits to the patients including ovarian cancer risk reduction, reduced infective morbidity following hysterectomy and contraception. The concept of OLS offers an opportunity for training in an essential gynaecological procedure in an 'in vivo' setting. It would be vital to garner consensus prior to this significant step. This study explores the attitude of professionals toward OLS.
Methods
A survey was undertaken at five hospitals, recruiting trainees & consultants, theatre personnel and pathologists. The questionnaire permitted free text commenting.
Results
The response rate was 100% amongst 150 participants. Majority of the consultants (90%) and theatre personnel (100%) support the concept of OLS for training. Trainees reported, OLS would offer additional training benefit (Median score of 8/10). 46% of participants felt OLS for training may have adverse effect on the patients. 70% of participants felt further ethical exploration amongst patients would be warranted. 92% felt trainees should undertake prior simulation-based training. 91% of trainees had used a simulator with 37% having performed a simulated salpingectomy; the stage of training did not significantly influence the latter. 44% of theatre staff expressed OLS may impact on theatre work flow. 100% of consultants and pathologists recommend histological assessment of the surgical specimen.
Discussion
The clinic-pathological argument for OLS is compelling. OLS offers a unique training opportunity. The concept of OLS training could be enhanced through simulation based training. This would introduce the 'pre-trained' novice to the 'in vivo' training using OLS. This may resolve any ethical and safety concerns of OLS.

FC15- Laparoscopic Myomectomy for Large Myomas

Danai Balfoussia[1*], Lindsay Kindinger[1], Hua Zen Ling[1], Tom Setchell[1], Tariq Miskry[1]
[1]St Mary's Hospital, Imperial College Healthcare NHS Trust London UK

Introduction
Laparoscopic myomectomy has established itself as an alternative to open myomectomy. Its role in the setting of larger fibroids has been controversial due to technical challenges. We present a case series of laparoscopic resection of fibroids ≥8cm in our institution

Methods
This was a single-centre retrospective review of patients undergoing laparoscopic myomectomy for large fibroids (≥8cm) between September 2005 and December 2014. Outcomes included operative time, complications, blood loss and rate of conversion to laparotomy.
Results
One hundred and forty nine patients aged 25-64 years (median: 38) underwent laparoscopic surgery. The commonest symptoms were menorrhagia (29%) and pressure effects (28%). One hundred and fifty leiomyomas were removed, ranging between 8-20cm (median: 10cm) and weighing between 76-1600g (median: 450g). Operative time ranged from 55 to 300 minutes (median: 120 minutes) and 56 patients (38%) had a concurrent procedure. Blood loss was 20-2000mls (median: 150mls). Two patients underwent laparotomy for specimen retrieval. Five patients required blood transfusion. One patient had a pulmonary embolism. A single patient required laparoscopy for small bowel obstruction four weeks post surgery. Finally, one patient developed a collection and one an ileus. Both were managed conservatively.
Conclusion
This is the largest UK case series examining laparoscopic myomectomy for large myomas (≥8cm). Our findings demonstrate that in experienced hands a laparoscopic approach should not be limited by fibroid size per se.

FC16- A warm-up strategy is effective in reducing mental load during laparoscopic prophylactic bilateral salpingo-oophorectomy: A Randomised Controlled Trial

Rasiah Bharathan[1,2*] James McLaren[1], Thomas Ind[1]
[1]St.Georges Hospital London UK; [2]Maidstone and Tunbridge Wells Hospitals NHS Trust Tunbridge Wells UK

Introduction
Laparoscopic prophylactic bilateral salpingo-oophorectomy (LapBSO) is performed as a risk reduction intervention for ovarian cancer. Pre-task warm-up is proven to enhance performance. This is the first trial to explore mental load during surgery.
Method
This is a cross-over randomised trial. Participants were stratified prior to computer generated allocation. Eighteen participants were first trained to proficiency bench. Training and assessments were completed on LAP Mentor virtual reality simulator. Each participant performed a 'control' LapBSO and a warm-up task followed by LapBSO. The warm-up task was 'circle cutting' which is FLS validated; this is time-limited to five minutes. During the LapBSO the participants were required to simultaneously perform a validated visuo-cognitive secondary task. After the LapBSO tasks, participants completed two validated questionnaires - NASA-TLX and subjective mental effort questionnaire (SMEQ).
Results
Warm-up intervention lead to significant reduction in SMEQ scores (P=0.02). In n four of the six dimensions of NASA-TLX, warm-up intervention resulted in significant improvement in workload measures [mental demand (P=0.04), temporal demand (P=0.015), performance (P=0.007) and frustration (P=0.003). The ratings approached statistical significance for physical demand (P=0.051) and effort (P=0.06).
The visuo-cognitive secondary task measure of mental load, revealed a significant reduction in mental load: the overall detection rate (P=0.003) and correct detection rates (P<0.05) were significantly higher in the interventional arm.
The correlation coefficient between mental load and SMEQ is significant for the control group (0.801) and interventional group (0.72); this strengthens our findings.
Conclusion
Pre-task warm-up is an effective technique in reducing mental load during LapBSO.

FC17- The effect of co-morbidity on the cost of laparoscopic surgery for endometrial cancer

Selina Chiu[2,1*], Michelle Harris[1], Marielle Nobbenhuis[1], Thomas Ind[1]
[1]*Royal Marsden Hospital London UK*; [2]*Aberdeen University Aberdeen UK*

AIMS
To determine the effects of co-morbidities such as obesity, diabetes, hypertension and age on the in-patient costs of laparoscopic endometrial cancer (EC) surgery.
METHODS
Seventy seven patients with EC treated by laparoscopic surgery were assessed. Clinical data were obtained from the trusts electronic patient record (EPR). The costs of each in-patient episode were calculated independently by the trust's finance department and included the costs of staff, ward, theatres, drugs, intensive care, rehabilitation, pathology, imaging and blood products.
RESULTS
Diabetes was associated with an increased median cost of £2426.83 (P = 0.0385). Hypertension, age over 65, and a Body Mass Index (BMI) of over 30 were associated with median increased costs of £2070.66 (P = 0.0044), £1842.40 (P = 0.0084) and £1699.59 (P=0.0225) respectively. No statistical difference was demonstrated in median costs for women who had had a previous laparotomy.
CONCLUSION
Diabetes, obesity, hypertension and increased age are associated with a significant increased cost of surgery for EC. This should be reflected

FC18- Evolution in the surgical management of endometrial cancer (2007-2014) at the DELTA centre - Royal Derby Hospital

Natalie Grant[1*], Graham Geary[1], Rahamatulla Latheef[1], Summi Abdul[1], Royal Derby[1]
[1]Hospital Derby UK; [2]*DELTA Centre Derby UK*

Aims: Assess the changing management of endometrial cancer 2007-2014.
Methods: Patient records attained from cancer database. Data collected retrospectively from electronic records.
Results: 618 patients assessed. Age-mean=66 (28-92). Pre-operative investigations: endometrial thickness-mean=14.95mm (1-85mm), uterus AP diameter-mean=39.4mm for TLH (13-73mm), 44.6mm for TAH (20-96mm). Pre-op hysteroscopy 65.2% cases. Histology-88.1% endometrioid, serous 3.81%, carcinosarcoma 6.68%, other 1.41%. Management in 2008: TLH 15.9%, TAH 62.3%, LAVH 18.8%. In 2014: TLH 74.7%, TAH 22.1%, LAVH 1.1%. 40 laparoscopic cases combined with PLND. Operating time-mean: TLH 97.99 minutes(36-213), TAH 108.13 minutes(44-250), (P=0.0071). Estimated blood loss-mean:TLH 212ml, TAH 469ml (P<0.0001). Length of stay-mean: TLH 2 days, TAH 5 days (P<0.0001). Rate of conversion (TLH to TAH): 5.44%(overall) - declined 17.5% 2008 to 3.76% 2013-2014. Reasons for conversion: bleeding, adhesions, failed entry, uterus too big and other. Complication rate: TLH 8.9%, TAH 16%. Serious complications <1%. Rate of readmission: TLH 7.96% (commonly vault haematoma), TAH 6.54% (commonly wound dehiscence/infection).
Conclusions: In our large 8-year case series there has been a significant change in surgical management from open to laparoscopic surgery, resulting in significantly shorter operating times, hospital stays, and lower morbidity. TLH can increase bed capacity allowing more cases to be done. Increased morbidity with TAH may be due to the more complex cases over TLH (bigger uterus, higher stage disease). All centres should offer TLH as a gold standard for all hysterectomies. The DELTA centre has developed a Training Programme for consultants and senior trainees to facilitate a transition in practice in other centres.

FC19- 'Laparoscopic Hysterectomies in the obese: not so dangerous after all'

Amy Hawarden[1*], Jeremy Hawe[1], Michael James McCormack[1]
[1]*Countess Of Chester Hospital Chester UK*

Rising BMI is a growing problem, with the current UK average BMI for women being 27. Initially, morbid obesity was considered a contraindication to laparoscopic hysterectomy, however in advanced laparoscopic units it is now considered an indication for, rather than a contra-indication to the laparoscopic approach.
417 cases of laparoscopic hysterectomies were performed between 2010 and 2015 at the Countess of Chester Hospital. The average BMI was 29. Of these patients, 40 were identified as having a BMI of 40 and above. These patients had a higher incidence of co-morbidities such as diabetes, hypertension and mobility issues and were more likely to have a pre-cancerous or cancerous indication for surgery (50% vs 29%). BMI ranged from 40-68, operation times (knife to skin) were longer (122mins vs 103mins), with a longer length of hospital stay (43 hours vs 40 hours), higher rates of intra-op (2.5% vs 1.3%), post op (7.5% vs 2.6%) complications, and a higher rates of re-admissions (2.5% vs 2.1%). Major complications included a thermal bowel injury requiring laparoscopic minor bowel resection, and a patient on anti-coagulant therapy requiring a blood transfusion after re-admission following vaginal bleeding.
Laparoscopic hysterectomy in women with a BMI > 40 is technically possible, with no intra-operative conversions to open, but is associated with an increase in morbidity compared to women with a lower BMI. However, most patients still benefit from the laparoscopic approach as opposed to an open procedure. Laparoscopy should be considered the preferred route for surgery in this group of women.

FC20- Day Case Laparoscopic Burch for Genuine Stress Incontinence

Alison Micallef Fava[1], Daliso Chetcuti[1*], Joy Got[1], Sarah Sultana Grixti[1], Igor Knyazev[1], John Mamo[1]
[1]*Department of obstetrics and gynaecology Mater Dei hospital Msida Malta*

Introduction: Surgical management is available for those select patients with genuine stress incontinence, when conservative measures are unsuccessful.
Aim: Comparison of results in patients undergoing open and laparoscopic Burch colposuspension, as well as mid urethral tension free mesh for the surgical management options of genuine stress incontinence.
Method: Patients who presented with stress incontinence underwent Urodynamics studies prior to undergoing a surgical intervention. Laparoscopic Burch colposuspension, open Burch procedures as well as mid-urethral tape procedure between 1st Jan 2013 and 31st Dec 2014 were included in the study. The length of stay following the respective surgical intervention as well as the readmission rate were assessed.
Results: Fourteen patients underwent laparoscopic Burch procedure (age range between 37 to 72years) while fifteen patients underwent open Burch procedure (age ranged between 40 to 62years). Mid-urethral tape procedures was carried out on twenty patients. (age ranged from 37 to 71years). The average length of stay was 2.57days (ranging from 1-3days), 6.22 days (ranging from 4-9days) and 2.95 days (ranging from 1-9days) for laparoscopic Burch colposuspension, open Burch colposuspension and mid-urethral tape procedure respectively. There were no readmissions after laparoscopic Burch colposuspension while there were two readmissions after open Burch colposuspension and two readmissions after mid-urethral tape procedures.
Conclusions: Patients undergoing laparoscopic Burch procedure had a shorter hospital stay, quicker recovery and earlier return to work; as well as requiring no readmissions. It is envisaged that we achieve day case laparoscopic burch colposuspension for the management of genuine stress incontinence.

FC21- Feasibility of A Randomised Controlled Trial (RCT) to compare Recovery, Pelvic Floor and Sexual Function following Laparoscopic Total Hysterectomy with that following Laparoscopic Sub-Total (Supracervical Hysterectomy): The LaHoST study

Hasib Ahmed[1,2*], Hany Wisa[1,2], Jeremy Wright[3]
[1]*Medway Maritime Hospital Kent UK;* [2]*Canterbury Christ Church University Kent UK;* [3]*University of Surrey Surrey UK*

Background: Patient benefit following ~~cervical~~ conservation at abdominal hysterectomy remains unproven (Lethaby 2012). Numerous observational studies suggest an advantage of Laparoscopic Supracervical Hysterectomy (LSH) over Laparoscopic Total Hysterectomy (LTH). The only randomised comparison showed no difference (Morrelli 2007) but assessments may have been too infrequent to demonstrate a difference.
Aim: To assess the feasibility of an RCT comparing recovery, pelvic floor and sexual function following LSH with that following LTH.
Method: Premenopausal women with a benign indication for hysterectomy, uterine size of less than 16 weeks gestation and less than 2nd degree uterine descent were randomised to LSH or LTH. Participants were followed up weekly using validated recovery questionnaires until 12 weeks. In addition they were asked to complete validated pelvic floor and sexual function questionnaires at baseline, 6 weeks and 6 months.
Results: 50 of 70 eligible women agreed to randomisation. Data collection was complete at 24 months. 100%, 88% and 60% of recovery questionnaires were captured at baseline, 6 weeks and 12 weeks respectively. Recovery data between 7 and 12 weeks did not appear discriminatory. 98%, 90% and 76% of pelvic floor and sexual function questionnaires were captured at baseline, 6 weeks and 6 months respectively. Recovery to normal activity occurred at 4-5 weeks in the LSH group compared to 7-8 weeks in the LTH group.
Conclusions: An RCT to compare outcomes following LSH and LTH appears feasible. Those undergoing LSH appear to be back to normal 2 weeks prior to those undergoing LTH. No effect was observed on bladder, bowel or sexual function. We propose a larger multicentre study to investigate this important issue.

FC22- Outcome of reproductive surgery in sub fertile women with tubal disease

Nihal Abdu[1*], Shamma Al-inizi[1]
[1]*South Tyneside NHS Foundation Trust South Tyneside UK*

Tubal disease accounts for at least 25% of female factor subfertility with more than half of the cases due to Chlamydia salpingitis. We have reviewed our reproductive surgical outcome in a group of 89 sub fertile women who underwent laparoscopic tubal surgery having had tubal co morbidities such as previous Chlamydia infection (75%) or endometriosis (25%). Tubal disease was graded laparoscopically.
The commonest surgical interventions undertaken were division of adhesions/ tubal surgery and HELICA coagulation of endometriosis. Occasionally, division of large hydrosalpinx, proximal tubal cannulation or total salpingectomy to prepare for IVF were performed. After the surgery, 51% of the patients who had Chlamydia induced tubal disease were referred for IVF indicating the significant damage this organism might cause to the fallopian tubes; and only 31% of those with negative Chlamydia serology needed IVF referral. The rest of the patients received either ovulation induction or had conceived naturally. Pregnancy rate was 40% in women who had Chlamydia induced tubal disease (3 had ectopic pregnancy) and 38% in those with negative Chlamydia serology. Although the recommendation for tubal disease favours assisted reproductive technique (ART), still tubal microsurgery has the advantage of long-standing restoration of fertility. The NICE Guideline favours laparoscopic tubal surgery for mild tubal disease and salpingectomy for hydrosalpinx before ART. Adequate counselling regarding the risks of surgery and ectopic pregnancy is paramount.
Proper laparoscopic grading of tubal disease with adequate counselling following consideration of clinical picture and patient`s preferences can result in a good success rate.

FC23- Combined laparosciopic ovarian tissue cryopreservation and retrieval of immature oocytes followed by in vitro maturation and vitrification: Results from the Oxford Ovarian Tissue Cryopreservation (OTCP) Programme

Christian Becker[1,2*], Enda McVeigh[1], Tim Child[1,3], Karen Turner[4,3], Kokila Lakhoo[5], Muhammad Fatum[1,3]
[1]*NDOG, Oxford University Oxford UK;* [2]*Oxford Fertility Unit Oxford UK;* [3]*Institute of Reproductive sciences Oxford UK;* [4]*Oxford Heart Valve Bank Oxford UK;* [5]*Oxford Children's Hospital Oxford UK*

Introduction: Combining ovarian tissue cryopreservation with retrieval of unstimulated immature oocytes followed by IVM and vitrification may potentially increase the prospects of fertility preservation in oncologic patients. No clear consensus is established whether a combined strategy should be utilized.
Aims: To study the feasibility of intraoperative egg retrieval in oncologic patients undergoing ovarian tissue preservation. The different surgical techniques utilized to be described.
Materials and Methods: A retrospective study of the first clinical ovarian tissue cryopreservation(OTCP) service in England. Oncology patients undergoing OTCP before high-risk gonadotoxic chemo-radiotherapy, were all offered immature egg retrieval, IVM and vitrification. Egg retrieval was done either by *ex situ* puncturing the ovary after excision or by percutaneous video-assisted oocyte pick-up in patients undergoing ovarian cortical strips resection, in addition to the fluid collection after tissue processing.
Results: The results of the first two years of activity at Oxford University OTCP Programme will be presented. . So far, 20 patients aged 2-31 years were recruited and undergone laparoscopic ovarian tissue harvesting and *ex situ* or *in situ* video-assisted egg retrieval. In 14/20 patients immature eggs were retrieved. The youngest patient with viable oocytes found, was 9 years old. Maturation rates and correlations will be presented. No major adverse events were experienced.
Conclusions: Oocytes can be retrieved by *ex-situ* puncturing of the excised ovary, *in situ* percutaneous video assisted egg retrieval during laparoscopy or from the processing fluid, then matured *in vitro*, and cryopreserved by vitrification. This fertility preservation modality could be combined with ovarian tissue preservation.

FC24- How to interpret presenting symptoms of ectopic pregnancy? The triad associated with major haemorrhage

E Mullins[1], D Chandrashekar[1], N Agarwal[1], F Odejinmi[1]
[1]*North West London Hospitals NHS Trust London UK;* [1]*Barts Healthcare NHS Trust London UK*

Introduction
Ectopic pregnancy (EP) occurs in up to 2% of pregnancies and can be life threatening (Barnhart 2009). The association between presenting symptoms and quantity of intra-peritoneal bleeding has not been assessed.
Methods
Prospective audit of all women requiring surgery for EP over a 5-year period at Whipps Cross Hospital. Secondary analysis of presenting symptoms and blood loss documented on the operation note was performed. We analysed the association of presenting symptoms with blood loss at surgery. Statistics were calculated with SPSS v.20, Mann-Whitey U-test was used to compare groups, significance at $p<0.05$.
Results
318 women underwent surgery for EP during the 5 year study period, Table. 1.

Symptom	n= (%)	Median age	p=	Weeks amenorrhoea	p=	Median EBL at surgery (mls)	Range	p=
Any	318 (100%)	30.7	-	6.5	-	425	0-4200	-
Vaginal bleeding	294 (9.2%)	30.7	0.101	6.5	0.917	416.0	0-4200	0.485
Abdominal pain	310 (97.4)	30.8	0.094	6.5	0.673	422.0	0-4200	0.359
Diarrhoea	3 (0.9%)	31.6	0.892	6.3	0.892	1200.0	0-3000	0.416
Vomiting	24 (7.5)	31.0	0.638	6.3	0.723	1293	0-4000	**<0.0001**
Shoulder tip pain	30 (9.4%)	32.5	0.22	6.6	0.688	1671.0	100-4000	**<0.0001**
Syncope	23 (7.2%)	31.6	0.603	6.6	0.65	1854.0	100-4000	**<0.0001**

Statistical comparison of group of patients with the stated symptom to those without it

EBL= estimated blood loss

Table. 1. Characteristics and blood loss at surgery in women with the stated symptom at presentation

The triad of vomiting, shoulder-tip pain and syncope were associated with significantly increased blood loss at surgery (Fig. 1), none was associated with increased blood loss when independent of the other two. Shoulder tip pain and syncope combined (median EBL 1520mls (0-2800), p<0.0001) and the triad combined (median EBL 2562mls (500-4000), p<0.001) were associated with significantly increased blood loss.

Conclusion- Vomiting and syncope are signs of hypotension, shoulder-tip pain signals diaphragmatic stimulation, in EP likely due to intra-peritoneal haemorrhage. Their association with greatly increased blood loss may not be appreciated by clinicians. Those managing women with EP and shared CEPOD lists must understand the necessity of urgent surgery and blood products for women with suspected or confirmed ectopic pregnancy with vomiting, shoulder-tip pain or syncope.

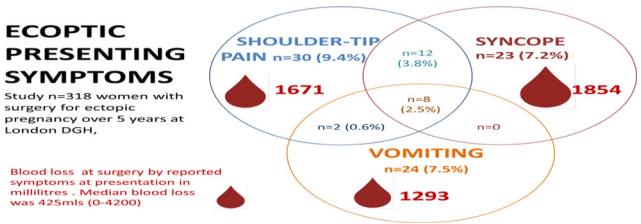


Figure 1. Summary Venn infographic

FC25- A Proposed Inexpensive Uterine Hysteroscopy Model for simulation-based Education

Somaia Elsayed[1*] Wei Zian Szetho[1], Ray O'sullivan[1], Naveen Usman[1]
[1]*St. Luke's General Hospital Kilkenny Ireland*

BACKGROUND: In obstetrics and gynaecology practice, hysteroscopy is the standard procedure for diagnostic evaluation of the uterine cavity and for operative treatment of uterine abnormalities.[1,2] Diagnostic and operative hysteroscopy can be challenging and requires significant skill set and good hand-eye coordination. Simulation can benefit the trainee, educator and patient. Simulation allows for practice, real-time feedback, and learning in a safe environment.[3] A growing body of data supports the effectiveness of formal, objective teaching of surgical skills and the effectiveness of surgical simulation in training.[4] Additionally, for some clinical tasks, simulation training can be effective when inexpensive but realistic models are used.[5] However there is a relative paucity of simulation-based education in obstetrics and gynaecology training programmes. Besides, the existing simulators are expensive and trainees have limited access to them due to lack of resources.

OBJECTIVE: We propose an effective, inexpensive and reproducible model for developing the skills and hand-eye coordination for diagnostic and operative hysteroscopy.

METHOD: Advanced surgical skills can be practiced and improved using simple clay, play-doh uterine models. Four different clay, play-doh uterine models were developed, demonstrating techniques of diagnostic and operative skills including removal of mirena coil, resection and biopsy of endometrial polyp and cannulation of fallopian tubes. A rigid hysteroscope is simulated Using flexible snake scope camera, while a pipette was used to simulate a channel for operative hysteroscopy.

CONCLUSION: The described play-doh uterine models are a cost effective way to improve hand-eye coordination in the use of diagnostic and operative hysteroscopy.

Video Poster Presentations

FCVP1- 'Pockets and Pouches': Dangerous, Deceptive Endometriosis:

Rajiv Sreekumar[1*], Dominic Byrne[1]
[1]*Royal Cornwall Hospitals Truro Cornwall UK*

Severe endometriosis can sometimes be very discrete and easily missed without a systematic thorough laparoscopic pelvic survey. Lesions can be hidden from view in a peritoneal pocket, or the pocket itself can be hidden behind other structures. This can lead to false negative laparoscopy or incomplete excision of disease.
Endometriosis can also cause subtle distortion of pelvic anatomy which on initial inspection can be unrecognised. Failure to recognise subtle bowel adherence creating a pouch can lead to major surgical complications. We present videos of 2 cases showing these pockets and pouches.
The first video demonstrates a significant endometriotic nodule situated deep within a peritoneal pocket in the recto-vaginal septum hidden from view behind the uterosacral ligaments. The video shows resection of the nodule by inverting the pocket to ensure complete excision of the disease.
The second case was referred for excision of "mild" endometriosis. There is subtle but significant tethering of the rectum creating a pouch up to the back of the uterus, which was previously missed at the first laparoscopy. The video shows release of the rectal tethering and the techniques employed to carefully resect the disease off the rectum. These two examples clearly demonstrate the misleading appearance of endometriosis and the traps that await both diagnostic and therapeutic laparoscopic surgery. Valuable lessons for us all.

FCVP3- VIDEO - Our first attempt at in-bag power morcellation

Thomas Ind[1,2], Owen Heath[1], Tim Hookway[1]
[1]*St Georges Hospital London UK;* [2]*Royal Marsden Hospital London UK*

Power morcellation has received a bad press recently due to the perceived risk of dissemination of inadvertant leiomyosarcomas.
A number of authors have advocated 'in-bag' morcellation.
This video demonstrates our first attempt to achieve in-bag morcellation of an ovarian fibroma. Particular attention is made on the methodology and difficulties encountered.
Overall, the procedure prolonged the operation by half an hour but we did not encounter many technical difficulties.

FCVP4- Teaching an old dog new tricks - A surgeon's first docking of the new Da Vinci Xi

Thomas Ind[1*], Ilyas Arshad[1], Marielle Nobbenhuis[1]
[1]*Royal Marsden Hospital London UK*

This is an unedited 10 minute video with sound. When a new Da Vinci Xi was purchased in our institution, one robotic surgeon had formal teaching in docking while another was taught in-house by the trained surgeon.
This video demonstrates how the Xi is docked in a real surgical setting and is an insight for those unfamiliar with robotic surgery. The video also shows the process of an established robotic surgeon being taught the new process.
It is clear from this video that even in a teaching setting, the process adds only ten minutes to the operation.

FCVP5- Extra-peritoneal laparoscopic colposuspension (EP-LC) for women with Stress Urinary Incontinence

Shaimaa Ibrahim[1*], Inna Sokolova[1], Robert Hawthorn[2], Wael Agur[1]
[1]*University hospital Crosshouse Kilmarnock/Ayrshire UK;* [2]*Southern general hospital Glasgow UK*

Introduction
Laparoscopic colposuspension has been shown to be equivalent to the open procedure in Cochrane reviews (1), however, it did not catch momentum due to the technical demand of the procedure the emergence of tension-free vaginal tapes (2).
Objective:
We present short-term follow up of the first 16 procedures performed by a simplified extraperitoneal approach.
Method:
Veress needle is introduced above the symphysis to insufflate 1L of CO_2 into the retropubic space (RS). Umbilical trocar introduced and rectus sheath is pierced midway between the umbilicus and the symphysis pubis. Recti are separated and the 'cob-web' of gas will guide the scope to RS. Two 5-mm trocars are introduced 2 cm above and 1 cm lateral to pubic tubercle. Using the usual perineo-abdominal approach, the vagina at the level of the bladder neck is dissected and attached to Cooper's ligaments with 2 non-absorbable sutures on each side. Straight needles with integrated knot-pusher are used. Data were extracted from BSUG national database.
Results:
16 procedures were performed over 18 months. 11/16 had pure and 5/16 had mixed incontinence. Mean BMI: 30.5 and mean age: 45.5. One patient required repair of bladder injury via laparotomy with no consequences.
3-month postoperatively, 15 patients were dry and 1 had persistent SUI. One patient developed de novo urgency and one required rectocele repair.
Conclusion:
EP-LC procedure appears to be a valid minimally-invasive alternative for women with SUI.
References:
1- Cochrane review. July 2006.
2- BJOG.2006; 113; 985-987.

FCVP6- A case of Ligasure failure to seal ovarian vessels in a patient with a history of ovarian vein emobilization during performing hysterectomy

Osama Eskandar[1*], Afaf Diyaf[1]
[1]*North Devon Hospital Barnstaple UK*

Case report
A 48 year old lady had a total laparoscopic hysterectomy for heavy and painful periods.
During the operation a Ligasure was used as an energy source to perform the hysterectomy. The infundibulopelvic ligament was cut successfully however; down near the anastomosis of the ovarian vessels with the uterine artery, the Ligasure repeatedly failed to initiate energy. It was thought that the Ligasure was faulty as the generator indicator kept illuminating indicating that the tissue has not been sealed. A closer look at the ovarian blood vessels, a spiral metal wire was found near the left pelvic side wall which was coming from the ovarian vein. The wire was removed laparoscopically and the rest of the operation was performed successfully.
From the history, this patient had had peripheral vascular disease. She had pelvic venography and left ovarian vein embolisation. As a part of the procedure, segment of titanium spiral wire is placed and left within the blood vessel.

Discussion
The Ligasure fails to seal vessels when the tissue impedance is out of range, the seal cycle was interrupted before the cycle was complete, insufficient amount of tissue inside the jaws of the ligasure or grasping metal objects; such as staples, wire, spiral, or clips; in the jaws of the instrument.
Conclusion
The clinicians should be aware that one of the reasons of the ligasure failure is a presence of metal object such as titanium wire between its jaws which can be hiden inside a blood vessel as in this case.

FCVP7- Introduction of a novel approach to maintaining Pneumoperitoneum at Total Laparoscopic Hysterectomy (TLH)

Lorna Hutchinson[1], Sirkhar Sircar[1], David McMurray[1], Karina Datsun[1*], Mohammed Allam[1]
[1]*NHS Lanarkshire Wishaw UK*

During TLH diathermy is used to circumferentially cut around the uterine manipulator. At this stage CO_2 can be lost.
We have developed a fluid filled "donut" which can be placed inside the vagina. This maintains pneumoperitoneum at the time of removing the uterus from the vagina. Once the specimen has been removed the "donut" can then seal the lower vagina while suturing takes place in order to avoid loss of CO2.
The following equipment is required:

• condom
• silastic foley catheter N14
• 60ml syringe
• suture material for tying - silk 0/2
• Sterile water

1. The catheter is inserted inside the condom (3-4 cm of the catheter tip should be placed just inside the condom)
2. Silk is used to tie a knot about 1cm from the end of the condom so it is fixed to the catheter
3. The tip of the condom should be then be placed alongside the above knot.
4. The tip of the condom is tied in place using the suture material forming a donut
5. The integrity of the donut is checked by filling it with 60ml of sterile water via the catheter.
6. Following insertion of the uterine manipulator the donut isthreaded over the handle of the manipulator and pushed up the vagina close to the cup.
7. Prior to excising the cervix the assistant inflates the donut with sterile water.
8. Following removal of the specimen the donut is replaced in the lower vagina to ensure ongoing pneumoperitoneum.
https://vimeo.com/122824001

FCVP8- Hysterectomy for Filshie clip Migration

Rebecca Hardcastle[1,2*], Chris Guyer[2]
[1]*Ethicon London UK;* [2]*University Of Surrey Guildford UK*

Objective
To examine an example of rare Filshie clip migration.
Setting
The Filshie clip has become the most popular sterilisation device in the UK since its introduction in the 1980's[2]. There have been a number of case reports published describing migrated clips[3,4,5] and it has been suggested that migration can occur in over 20% of cases[6].
Case:
Following an uneventful laparoscopic Filshie sterilisation a 36 year old P3 was seen in clinic (by multiple different consultants) complaining of pelvic pain. Investigations including a diagnostic laparoscopy gave a high suspicion of an embedded Filshie clip between the vagina and bladder. Follow up appointments were missed. Re-referrals lead to multiple clinicians' involvement. There was no note of the surgical findings and emphasis on pelvic pain occurred. She eventually had a TAH BSO.
She was re-referred, seeing a different consultant, with swelling in the vagina and a suggested foreign body between the vault and bladder on MRI/USS. Cystogram confirmed no communication to the bladder.
The patient had a n excisional laparoscopy, which identified a nodule containing an old abscess and a Filshie clip was retrieved from within.
Discussion
There was discontinuity of the patient care that has become common practice since the NHS plan[7]. This has led to the abandoning of named consultants and instead the use of clinician pools. The result is loss of clinical information and incorrect diagnostic pathways taken. It seems likely that, had the patient returned to the consultant performing the initial laparoscopy, perhaps hysterectomy would have been avoided.

FCVP9- Prophylactic skeletonisation of infundibulopelvic vessels and temporary clipping of bilateral uterine arteries during myomectomy for large sub mucous fibroid

Suruchi Pandey[1*], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK, Chertsey, UK*

Introduction
Randomized trials have demonstrated that laparoscopic myomectomy is associated with decreased morbidity and quicker recovery. However, it can be associated with considerable blood loss. Temporary clipping of bilateral uterine arteries is shown to decrease blood loss during myomectomy without affecting uterine perfusion. In addition, prophylactically skeletonising infundibulopelvic vessels and placing a loose knot around them can provide extra security to enable quick bleeding control if required.
Video Presentation
In our video presentation of a laparoscopic myomectomy for a large fibroid uterus, we demonstrate lateral pelvic sidewall dissection, ureterolysis, temporary clipping of bilateral uterine arteries, prophylactic skeletonisation of infundibulo pelvic vessels and myomectomy for a large fibroid uterus.

Following steps were undertaken:

1. Trocar placement was individualized to ensure easy manipulation
2. Pelvic survey was performed and bilateral ureters were identified
3. Right infundibulo pelvic vessels were skeletonised as these were particularly large. A loose knot was placed around the IP ligament prophylactically to be tightened in case of significant bleeding.
4. Peritoneum covering bilateral lateral pelvic sidewalls was opened and bilateral ureterolysis was performed
5. Uterine arteries were identified
6. Vascular clips were placed loosely over bilateral uterine arteries
7. Vasopressin was infiltrated over the myometrium covering the fibroids
8. Myomectomy and multiple layers of suturing were performed.
9. At the end of the myomectomy, bilateral vessel clips and temporary tie were removed
10. Haemostasis was reconfirmed after removing clips

Blood loss during surgery was minimal and the patient was discharged the next day.

FCVP10- Management of a large torted dermoid with a viable ovary: de-torsion with orchidopexy and interval cystectomy or de-torsion and cystectomy?

Suruchi Pandey[1*], Saikat Banerjee[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK, Chertsey, UK*

Introduction
Follow up of women of reproductive age who underwent de-torsion of torted ovaries has revealed resumption of ovarian function and successful pregnancies. Where there is a torted viable ovary with a large ovarian cyst, the choice is either to de-tort, perform orchidopexy and perform interval ovarian cystectomy or to de-tort and perform cystectomy to reduce the risk of re-torsion and repeat surgery.
Video Presentation
We present the case of a 30 year old with a known 12cm right dermoid. While awaiting elective surgery, she presented with classical signs of torsion. Prompt laparoscopy revealed that the cyst had torted 4 times around the utero- ovarian ligament. The ovary had a bluish hue but regained colour immediately after de-torsion. There was no obvious oedema. An uncomplicated ovarian cystectomy was performed but the cyst ruptured during the process. A thorough lavage was performed.
3 months after the procedure, the patient underwent a repeat laparoscopy for pelvic pain, which revealed a frozen pelvis, bilateral hydrosalphinges and an ovarian mass on the right side. Adhesiolysis, partial right oophorectomy and pelvic lavage were performed. Our differential diagnosis was: chemical peritonitis, pelvic infection post surgery and severe PID. The patient needed another pelvic lavage following which she recovered completely.
We present pictures and videos from the laparoscopic procedures and also discuss in detail the management of large torted ovarian cysts in women of the reproductive age group.

FCVP11- Video presentation of 2 different approaches for complex salpingo-oophorectomy

Victoria Asfour[1*], Saikat Banerjee[1]
[1]*St Peter's Hospital, London, UK*

Salpingo-oophorectomy is a common gynaecological operation. Increasing ovarian preservation at the time of hysterectomy, leads to more complex surgery to be required later in the woman's life. We present 2 different approaches to manage intraoperative access difficulties.
Case1: Frozen pelvis due to severe endometriosis. Unilateral salpingo-oophorectomy was performed for a large endometrioma in a patient with severe pelvic pain wishing for fertility. Ureteric stents inserted to facilitate identification at ureterolysis. Severe adhesions and fibrosis necessitated an unusual approach. Starting medially at the ovarian ligament and fallopian tube, ligating laterally. The ovary was stuck to sigmoid colon. The POD was obscured. The round ligament was divided, the ovary was medialised, the ureter identified and lateralised. The IP pedicle was divided last.
Case 2: Dense adhesions on entry were obscuring the pelvis due to previous hysterectomy.This patient was fully anticoagulated for cerebral vein thrombosis. We show a systematic adhesiolysis normalising the pelvis. Then, anatomical salpingo-oophorectomy is shown starting from the IP ligament. Once the pelvis was visualised, the ureter identified transperitonially. Meticulous haemostasis was achieved. Adhesiolysis and raw surfaces for dissection kept to a minimum. In this case ureter lateralised and IP ligament divided.
Conclusion: Laparoscopic surgeons need to build have a wide armamentarium of techniques to adapt to the challenges encountered intraoperatively safely.

FCVP12- Incidental finding of well differentiated papillary mesothelioma of the fallopian tube: a rare differential diagnosis of endometriosis at laparoscopy

Kenneth Ma[1*], Kingshuk Majumder[1], Rick Clayton[1]
[1]*St Mary's Hospital, Manchester, UK*

Well differentiated papillary mesothelioma (WDPM) is a mesothelial tumour that occurs in the peritoneum but rarely on the fallopian tube. A 36 year old nulliparous patient presented to gynaecology out-patients clinic with a 3 month history of lower abdominal pain, dysmenorrhoea and menorrhagia. Ultrasound scan found a complex ovarian cyst of the left ovary measuring 7cm in diameter. Magnetic resonance imaging suggest a 7cm endometrioma, adenomyosis and rectovaginal endometriosis. A plan for laparoscopic ovarian cystectomy and staging of endometriosis was arranged.
At laparoscopy the left fallopian tube had an unusal appearance, was swollen with mutiple psedocysts attached that was suggestive of endometriosis. A left salpingectomy, left ovarian cystectomy and excision of pelvic endometriosis was performed. Histology of the left fallopian tube showed well differentiated papillary mesothelioma while ovarian cyst wall and pelvic peritoneal biospy showed endometriosis. Our patient made an uneventful recovery and will now be followed-up with a interval diagnostic laparoscopy and perinteal biopsy to exclude multifocal disease.
WDPM is generally considered a tumour of low malignant potential, although little is known regarding its natural history and there are no consensus for its management. This case highlights its appearance at laparoscopy and the importance for histological diagnosis of endometriosis.

FCVP13- Unusual presentation of ovarian cyst, successful laparoscopic excision

Anita Nargund[1*], Anthony Griffiths[1]
[1]*UHW, Cardiff, UK*

We are here with presenting a successful laparoscopic excision of an unusually presenting dermoid cyst as a video presentation.
A 25 yrs old lady presented to Emergency unit with left side lower abdominal pain. She underwent extensive investigations, CT scan revealed large 14+12+19 cm cystic lesion in the upper quadrant, suggestive of dermoid cyst. Tumour markers were normal.
Ovarian cyst present in upper abdomen, mimicking mesenteric/splenic cyst.
As she was symptomatic, was offered laparoscopic excision of ovarian cyst with or without oophorectomy.
She underwent laparoscopy, cyst was removed without spillage into peritoneal cavity. Procedure was uneventful. Histology confirmed mature cystic teratoma.

FCVP14- Practical and anatomical advantages of uterine suspension for access optimisation in resection of deep infiltrative and rectovaginal endometriosis (video and animation)

Alexandros Derpapas[1*], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK*

Radical surgical excision of deep infiltrative endometriosis (DIE) is the current mainstay of treatment. Optimal surgical access is crucial for achieving good results. In our experience temporary suspension of the uterus, in addition to that of the adnexa, provides superior exposure than conventional uterine manipulation. We demonstrate the advantages of this technique via a short video and an animation that outlines the relevant anatomical relations.
The technique involves the passing of a 2-0 prolene suture on a straight needle through the skin suprapubically into the pelvis under direct vision. The needle is then passed through the uterine fundus in a dorsal to ventral direction and subsequently taken out through the anterior abdominal wall right by the original entry point. The suture is either tied extracorporeally over a Raytec gauze or clipped on a heamostatic clamp, so as adjustment of the degree of anteversion during the procedure is readily achievable. The same result can be achieved by a modified version of the technique, whereby a large curved needle is passed through the uterine fundus and the suture is retrieved through the skin by use of a rectus sheath closure device.
Temporary uterine suspension results in a better-exposed and still operating field due to avoiding excessive manoeuvres from the second assistant, especially when concomitant rectal manipulation is warranted. It is a

rather easy and quick method of enhanced exposure of the surgical field; hence we advocate its routine use where resection of endometriosis from the cul-de-sac and uterosacral ligaments is required.

FCVP15- Laparoscopic ventrosuspension as treatment for dyspareunia and dysmenorrhea: Another nice operation that doesn't work?

Alexandros Derpapas[1*], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK*

Mobile uterine retroversion has long thought to be the cause of pelvic pain symptoms. Various reports stemming mainly from uncontrolled cohort studies over the last 20 years have shown inconsistent results of surgical correction of acute uterine retroversion. More recent data from medium and long-term observational studies, however, suggest that despite some decline in the overall effect of surgical ventrosuspension with time, symptom improvement is durable in about 50% of cases.

Amid ongoing debate amongst laparoscopic gynaecologists about the efficacy of this method, we present two cases in which we performed laparoscopic ventrosuspension, hoping to reproduce the promising results reported in the literature. Both cases regarded premenopausal women presenting with severe persistent pelvic pain and no other intraoperative findings apart from an acutely retroverted uterus and elongated round ligaments.

A non-absorbable suture was placed at the most lateral aspect of each round ligament, at the point of its entry into the internal inguinal canal and tied laparoscopically. A running suture was continued medially, using several bites around the round ligament and tied to the short end of the initial lateral knot, resulting in shortening of both round ligaments and an axially positioned uterus.

No symptom improvement was evident at 3 months postoperatively in either of the cases. Although failure of this method in a small case series can not question the results in much larger cohorts, we remain unconvinced that correcting a finding that is present in around 15% of asymptomatic women can be an effective treatment for pelvic pain.

FCVP16- A failure of the Novasure Cavity Integrity Assessment (CIA) due to tubal patency: a video presentation. Dr Monika Oktaba, Mr Andrew Baxter, The Royal Hallamshire Hospital, Sheffield, UK

Andrew Baxter[1], Monika Oktaba[1*]
[1]*Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK*

A video will be presented demonstrating a failure of the Novasure Cavity Integrity Assessment (CIA) due to gas flow down a Fallopian tube. The ablation was being performed as part of a combined procedure and when the CIA would not pass it was noted laparoscopically that CO2 was flowing from the fimbrial end of one of the tubes. When this tube was clamped proximally the flow ceased and the CIA passed. A video demonstrating the sequence of events clearly will be presented and discussed. This potential problem is not mentioned in device literature. If this phenomenon is more than an isolated event it could lead to:

- trauma to the cervix in attempts to seal an 'incompetent' cervix
- unnecessary hysteroscopic checks of the cavity
- wasted devices
- if no other technique available, patients potentially having an unnecessary GA without an ablation being performed

It would be interesting to know the incidence of 'failed' Novasure procedures and whether this tubal cause of a failed CIA is a major contributing factor.

FCVP17- TITLE: A Junior Trainee: Laparoscopic salpingectomy for ectopic pregnancy

Elisabeth Bean[1*]
[1]*University College London Hospital, London, UK*

Developing skills for laparoscopy is an essential part of our training curriculum in Gynaecology. As an ST3 in Obstetrics & Gynaecology, with an interest in laparoscopy, I have been developing skills under senior supervision. Having achieved competence in diagnostic laparoscopy, I am now gaining confidence in simple minimal access procedures. I am in a privileged position training in a unit with regular laparoscopic lists, where consultants and senior registrars alike are highly experienced and confident in supervising juniors.

The most common laparoscopic procedure that trainees will be expected to do and should be competent to carry out independently by ST6 is a laparoscopic salpingectomy. It is a valuable skill if you are the senior presence out of hours, in order to treat the acute admission of an ectopic pregnancy, when your consultant may be delayed in attending.

I show two videos comparing two techniques, both useful in their own right.

1. Bipolar and cold scissors: Achieves full excision of the fallopian tube, without leaving a tubal stump. A favourite of reproductive medicine specialists, who fear stump ectopics in the event of an incompletely excised tube. However, often time consuming, with the need for frequent swapping of instruments and a potentially dangerous tool near neighbouring bowel.
2. Loop excision: a more simple but effective method. Enables quick haemostasis in the event of rupture or active bleeding and usually prevents the need for diathermy. An efficient method to allow the ever pressured registrar to return quickly to their duties on labour ward.

FCVP18- A video illustration of a proposed cost effective uterine hysteroscopy simulator

Somaia Elsayed[1*], Wei Zian Szetho[1], Ray O'Sullivan[1]
[1]*St Luke's General Hospital, Kilkenny, Ireland*

BACKGROUND: In obstetrics and gynaecology practice, hysteroscopy is the standard procedure for diagnostic evaluation of the uterine cavity and for operative treatment of uterine abnormalities.1,2 Diagnostic and operative hysteroscopy can be challenging and requires significant skill set and good hand-eye coordination. Simulation can benefit the trainee, educator and patient. Simulation allows for practice, real-time feedback, and learning in a safe environment. 3 A growing body of data supports the effectiveness of formal, objective teaching of surgical skills and the effectiveness of surgical simulation in training.4 Additionally, for some clinical tasks, simulation training can be effective when inexpensive but realistic models are used.5 , however there is a relative paucity of simulation-based education in obstetrics and gynaecology training programmes. Besides, the existing simulators are expensive and trainees have limited access to them due to lack of resources.

OBJECTIVE: We propose an effective, inexpensive and reproducible model for developing the skills and hand-eye coordination for diagnostic and operative hysteroscopy.

METHOD: Advanced surgical skills can be practiced and improved using simple clay, play-doh uterine models. This video describes Four different clay, play-doh uterine models, demonstrating techniques of diagnostic and operative skills including removal of mirena coil, resection and biopsy of endometrial polyp and cannulation of fallopian tubes. A rigid hystroscope is simulated Using flexible snake scope camera,while a pipette was used to simulate a channel for operative hysteroscopy.

CONCLUSION: The described play-doh uterine models are inexpensive, effective, reproducible and can be used to improve hand-eye coordination in the use of diagnostic and operative hysteroscopy.

FCVP19- Efficacy and safety of laparoscopic sacrocolpopexy for post hysterectomy recurrent vaginal prolapse

Abdalla Fayyad[1*], Damola Onifade[1], Ivlina Pandeva[1]
[1]*Luton and Dunstable University Hospital, Luton, UK*

Aim: To prospectively evaluate the efficacy and safety of laparoscopic sacrocolpopexy for the management of post hysterectomy recurrent vaginal prolapse
Methods: 160 consecutive women who underwent laparoscopic sacrocolpioopexy for recurrent post hysterectomy vaginal prolpase were prospectively evaluated over a 5-year period. Sacrocolpopexy was performed with a Y shaped mesh after dissection of the vagina from the rectum and the bladder. Patients were assessed at 3, 12 and 24 months using the Prolapse Quality of Life (P-QOL) questionnaire; Patient Global Impression of Improvement (PGII) and were examined using the Pelvic Organ Prolapse Quantification system (POP-Q).
Results: 88% of patients reported complete cure of vaginal bulge symptoms. 92% reported feeling "much better" or "very much better" on PGII. 15% had recurrent anatomical prolapse defined as point Ba ≥ -1, which were asymptomatic apart from eight patients (5%) that underwent further surgery. Postoperatively, vault support (point C) was at stage 0 in all patients. Two patient developed vaginal mesh extrusion that needed surgical revision.
Conclusion: Laparoscopic sacrcolpopexy with Y shaped mesh placement is safe and effective treatment for recurrent vaginal wall prolapse up to 2 years follow up. The procedure had minimum complications and should be considered the gold standard in recurrent prolapse.

FCVP20- Resection of large endometriotic nodule from bladder with full thickness bladder wall resection

Lidia Ewa Kwasnicka, MRCOG MSc[1*], Richard Penketh, BSc MD FRCOG[1], Shibs Datta, FRCS[1], Elizabeth Bruen, RGN[1]
[1]*University Hospital of Wales, Cardiff, UK*

A 27 year old underwent laparotomy by a gynaecologist and urologist elsewhere. Her bladder nodule was not tackled as it was felt by both surgeons to be too large to safely remove without diminishing bladder capacity.
At the age of 30 she was referred to the University Hospital of Wales, Cardiff, Endometriosis Centre for further management. She complained of severe dysmenorrhea, constant pelvic pain, menstrual haematuria, and cystitis like symptoms during and after her period. On cystoscopy she was found to have visible endometrioma sub mucosally above the right ureteric orifice and protruding into the lumen of the bladder.

Operative Laparoscopy

Following cystoscopy and bilateral ureteric stenting under X-ray guidance she underwent laparoscopic excision of bladder endometriosis.
The bladder dome was welded to the anterior uterine wall by a large endometriotic nodule occupying the utero-vesical fold. The extensive disease in her posterior compartment was not addressed on this occasion The nodule was bisected transversely using a monopolar hook. The bladder was then separated from the lower uterus. The remaining endometriotic tissue was shaved off the uterus, and bladder to leave healthy bladder tissue. The bladder was repaired with continuous 3/0 Vicryl suture. The patient was discharged the next day with an indwelling catheter which was removed 10 days later after a normal cystogram.
Ureteric stents were left in situ and will be removed following the second stage of her operation when her recto vaginal and pelvic endometriosis will be addressed.

FCVP21- Laparoscopic excision of rudimentary uterine horn with a failed pregnancy at nine weeks gestation

Kenneth Ma[1*], Jennifer Hemers[1], Roberta Morris[1] ,Kingshuk Majumder[1], Edmond Edi-Osagie[1]
[1]*St. Marys Hospital, Manchester, UK*

Rudimentary horn pregnancies are rare and have an estimated incidence of 1 in 76 000 to 150 000 pregnancies. We present a case of a failed rudimentary horn pregnancy at 9 weeks gestation managed by interval laparoscopic excision and morcellation.
A 34 year old Para 1 lady initially presented with a delayed miscarriage diagnosed on ultrasound scan outside of NHS services. Images on repeat ultrasound scan were suggestive of an ectopic pregnancy but at laparoscopy a rudimentary uterine horn pregnancy was diagnosed. A single dose of methotrexate was given in this case to reduce vascularity of the rudimentary horn before an interval procedure four weeks later to complete excision. Magnetic Resonance Imaging(MRI) was used to assess pelvic anatomy and demonstrated communication between both uterine horns. At surgery an advanced energy source was used for tissue dissection and intra-corporeal suture ligation was used to achieve haemostasis. The excised rudimentary horn was removed by morcellation and the fetus was retrieved separately intact.
This case highlights the need for a high index of suspicion for uterine anomalies at ultrasound scan and also demonstrates techniques common to other laparoscopic procedures. Our literature search found that although most rudimentary horn pregnancies have been managed by laparotomy in the past there is an increasing number of cases managed laparoscopically and this appears to be as safe and effective.

FCVP22- Demonstrating the advantages of 3D laparoscopy, the precision of operating in small spaces; case of severe endometriosis with complex adhesions due to previous midline laparotomy and caesarean section

Oudai Ali[1]
[1]*West Cumberland hospital, Whitehaven, UK*

Objective
3D laparoscopy needs to be appraised outside research laboratories. Its value in enhanced detailed visual navigation in narrow spaces is demonstrated in multiple adhesions and endometriosis.
Case
A 35y female with previous caesarean and long midline laparotomy while young to correct pyloric stenosis had long standing pelvic pain, dyspareunia and dysmenorrhea despite mirena inserted 3y ago. Examination indicated BMI of 32 and scarred abdomen from previous operations. Pelvic examination indicated tender bulky immobile retroverted uterus with scarred Douglas Pouch. Ultrasound and CT indicated Left complex 10x12cm adnexal mass towards the benign end of the spectrum with raised series of Ca125 but values less 200.Patient agreed for laparoscopic adnexectomy.
Methods and Results
Palmers point was used to introduce 10mm 3D Einstein vision technology scope. Findings indicated extensive adhesions creating difficult access to the pelvis which was scarred from endometriosis and previous caesarean and filled by left sided big endometrioma. Extensive adhesiolysis was performed with great accuracy and the added depth appreciation helped operating in confined spaces. The added feature of autonomous scope warming kept the view steady and avoided the interruptions to defog scope lenses. No difficulty was experienced by surgeons and staff in using the system and left salpingo-oophrectomy was achieved. Patient made

full recovery and discharged home after 24h and the follow up reported improved symptoms and return to normal function
Conclusion
In advanced laparoscopic gynaecology surgeons should consider 3D laparoscopy and adopt its benefits of improved vision and depth perception particularly in complex cases.

FCVP23- Laparoscopic Management of Residual Interstitial Pregnancy

Julia Kopeika[1*], Judith Hamilton[1], Kumar Kunde[1]
[1]*Guys and St Thomas NHS Hospital, London, UK*

Interstitial ectopic is reported in 2 to 4% of all ectopic pregnancies. The management can be challenging even where diagnosis is made in a timely manner, before potentially life-threatening rupture has occurred. Surgical management carries an increased risk of bleeding due to a high vascularity of interstitial part of the uterus. We encountered a case where repeat laparoscopy was required 4 weeks after incomplete removal of an interstitial ectopic pregnancy.
Methods: We demonstrate the laparoscopic management of an incompletely removed interstitial ectopic pregnancy.
Results: A patient presented with suboptimal rise of βHCG. Following an ultrasound diagnosis of tubal ectopic pregnancy, laparoscopic salpingectomy was performed. However, βHCG continued to rise with a maximum level of 12500iu/l following surgery. A repeat ultrasound scan showed presence of residual interstitial ectopic pregnancy. Patient declined medical treatment and opted to have a surgery. During the repeat laparoscopy, adhesions and postoperative inflammatory changes were found, making tissue more vascular and friable. However laparoscopic removal of residual trophoblastic tissue with intra-corporeal suturing of the uterus was successfully performed. The patient recovered uneventfully.
Conclusion: Laparoscopic removal of residual interstitial ectopic pregnancy is feasible and should be attempted. This video will demonstrate the steps that were undertaken to do this successfully.

FCVP24- Laparoscopic management of a ruptured interstitial pregnancy associated with massive haemoperitoneum and history of ipsilateral salpingectomy

Vasileios Minas[1*], Khadija Ahsraf[1]
[1]*Wirral University Teaching Hospital, Upton, Wirral, UK*

Interstitial pregnancy is an ectopic pregnancy which is implanted in the interstitial part of the fallopian tube, the part that transverses the myometrium. It accounts for 2-4 % of all tubal gestations. Mortality rates are reported as being between 2-2.5 %. The commonest risk factor is history of ipsilateral salpingectomy. Traditional treatment, particularly in cases of haemodynamically unstable patients, has been by laparotomy. Literature review reveals that a few such cases have been managed by laparoscopy and either cornual resection and suturing or suturing alone.
Our patient presented with low abdominal pain at 7 weeks gestation. She was para 1 with one previous caesarean section and a right laparoscopic salpingectomy for previous ectopic pregnancy. Her beta-hCG was approximately 6000 mIU/ml and her haemoglobin 13.5 g/dL. Ultrasound examination showed a left adnexal mass and small amount of free fluid raising the suspicion of a left-sided ectopic. She was scheduled for a laparoscopy. Over the following hours she became tachycardic and hypotensive, her haemoglobin dropped to 9.5 g/dL and the procedure was expedited. At laparoscopy massive haemoperitoneum was seen, the left adnexae appeared normal and a ruptured right interstitial pregnancy was diagnosed. The pregnancy tissues were removed and the uterine wound repaired with intracorporeal suturing. The estimated blood loss was 2.5 lt. Post-operative recovery was uneventful and the beta-hCG levels dropped rapidly.
In conclusion, ruptured interstitial pregnancy with massive haemoperitoneum may be managed safely by laparoscopy, provided the required skills and the option to quickly convert to a laparotomy are in place.

FCVP25- Diagnostic and Intra-operative Challenges for Ovarian Ectopic Pregnancy, Including Ovarian Conservation

Elisabeth Bean[1*], Kostas Doufekas[1], Ghada Salman[1], Alfred Cutner[1], Davor Jurkovic[1]
[1]*University College London Hospital, London, UK*

Up to three percent of ectopic pregnancies develop in the ovary, thought to be subsequent to fertilisation prior to ovulation, implantation on the ovarian surface or presence of endometriosis. They can be technically difficult to diagnose on ultrasound scan and may well be an unexpected finding at laparoscopy. There is some suggestion that the presence of an IUCD may be a pre-disposing factor and that they may be more likely when conception follows IVF treatment.
We show two cases of ovarian ectopic pregnancy, both diagnosed by pre-operatively on ultrasound and managed with conservation of ovarian tissue at University College London Hospital.

FCVP26- Laparoscopic CESA (CErvical SAcropexy) guided by retroperitoneal tunnelling: an anatomic reconstruction

Rasiah Bharathan[1*], Rahul Gore[1], Rowan Connell[1], Elias Kovoor[1]
[1]*Maidstone and Tunbridge Wells Hospitals NHS Trust, Maidstone, UK*

Case summary
A 56 year old woman presented with symptomatic stage 3 utero-vaginal prolapse.
Procedure
A laparoscopic subtotal hysterectomy was performed. Then a one centimetre peritoneal window was created over the S2 vertebral body on either side. A PVDF mesh is sutured onto the cervical stump using ethibond. A tunnelling device was inserted through one of the windows and advanced toward the cervical stump. The proximal end of one arm of the mesh is grasped by the tunnelling device and retracted through the retroperitoneal tunnel. This manoeuvre is performed on the contralateral side. These 8 cm arms form the *neo*-uterosacral ligaments. Then the proximal ends of the mesh is secured to the S2 body with ethibond. The peritoneal windows are closed.
Patient was discharged on day two. Post-operative follow up at 8 week revealed excellent anatomical support and functional results.
Discussion
We describe a laparoscopic modification of the traditional open sacropexy with the added advantage of a near normal anatomical support. The arms of the mesh comprise of non-absorbable sutures; not mesh. There are three mesh segments – a distal cervical segment and the two sacral components.
In view of the much small volume of mesh and a more anatomical support, we feel that this technique is safer than the traditional procedure.
A limitation of this technique is that it does not addressing the anterior or posterior compartment defects. In our experience, correction of the apical compartment obviates the need for further repair. Long term results are awaited.

FCVP27- A video illustration for morcelation in a bag Laparoscopic Supracervical Hysterectomy (LASH):

Manhal Najdy[1], K Elsapagh[1]
[1]*Wishaw General Hospital, Glasgow, UK*

Majority of hysterectomies are performed for benign conditions (***Einarsson JI, et al, 2009***).

Objective:
To assess the safety, reproducibility and cost efficacy of LASH. We compared the cost effectiveness of LASH and open subtotal hysterectomy and intruduced morcellation in bags towards the end of the audit.
Methods:
Data was collected retrospectively from 143 of women who underwent LASH from August 2008 - November 2014 for various benign indications with normal cervical smears and endometrium. Patients towards the end of the study had morcellation in bag in a way to comply with the recent FDA safety advice and it seems that operating time is getting better with learning curve.
Results:
3.4% had wound infection however only one pelvic haematoma, one ureteric oedema, one uterovaginal fistula, one hernia from lateral port site and one had scar pain at morcellator porte. No conversion to laparotomy, no blood transfusion, no DVT and no return to theatre. Average theatre time was 70 minutes for open subtotal hysterectomy and (90 min for LASH 15 min. for bag morcellation). The average cost per minute is £3.08.
Instruments for LASH costs £1156 plus cost of bag variable versus £200 for open. The average stay for LASH was 1.7 nights versus 3.7 nights for open. Average cost per night was £486. Overall cost of LASH was £2259.2 versus £2213.8 for open.
Conclusion:
LASH is safe, reproducible, cost effective and quicker recovery and less complication rates and can comply with FDA uterine morcellation advise.

FCVP28- A combined cystoscopic and laparoscopic approach to resect a full-thickness deep endometriotic nodule from the bladder

Kirana Arambage[1*], Horace Roman[2], Geoffroy Pasquier[2], Benoit Resch[2], Emmanuel Huet[2]
[1]*John Radcliffe Hospital, Oxford, UK; 2Rouen University Hospital, Rouen, France*

Setting: A French university hospital.
A 34-year-old nulliparous woman with a large (35-mm) endometriosis nodule infiltrating the bladder and deep endometriotic lesions of the rectum and sigmoid colon.
Intervention: The urological surgeon has performed cystoscopy to identify the limits of mucosal involvement, and incised the muscular layer up to the subcutaneous tissue surrounding the bladder. The gynecological surgeon identified and followed the circular incision, and completed full-thickness resection of the bladder wall to isolate large nodule. Surgical technique reports in anonymous patients are exempt from ethical approval by the institutional review board.
Measurements and Main Results: The patient's functional outcome was better. The laparoscopic resection of large endometriotic nodules of the bladder per se may lead to inadvertent removal of healthy bladder muscle. Thus, it increases the risk of postoperative complications and symptoms due to small bladder volume. Conversely, if resection of the nodule is performed only cystoscopically, it probably would not be completely removed. The combined approach enables to complete resection of the endometriotic nodule. It not only averts the risk of excessive removal of healthy bladder muscle but also leaves no disease behind.
Conclusions: On the basis of our experience, we propose the combined cystoscopic and laparoscopic approach in managing large endometriotic nodules with full-thickness infiltration of the bladder.

FCVP29- Very big cervical broad ligament fibroid, an intraoperative surprise during laparoscopic hysterectomy; a demonstration to deal with the unexpected finding, a strategy to manage retroperitoneal masses

Oudai Ali[1*]
[1]*West Cumberland Hospital, Whitehaven, UK*

Objective
It is recommended to exercise the best pathways in preoperative assessment to reduce intraoperative uncertainty before committing theatre resources and time. An unexpected big retroperitoneal mass was managed laparoscopically and retrieved vaginally.
Case
45y para 4 female presented with pain, pelvic mass and normal Ca125. Scans indicated 9cm right adnexal mass suggesting a big dermoid. On examination there was palpable mass at the right iliac fossa which was mobile and filled the Douglas Pouch. She opted for pelvic clearance given other symptoms of dysmenorrhea and dyspareunia.
Methods
Laparoscopy indicated rather a very big mobile retroperitoneal mass occupying the whole right broad ligament and consistent with cervical fibroid pushing the uterus to the left of the pelvis. The Vcare manipulator was actually in the fibroid rather than the uterus. Pelvis sidewall dissection was meticulous and achieved full mobilisation down to the origin of the fibroid. Hysterectomy was concluded laparoscopically and the specimen was retrieved vaginally after morcellation. Alexis retractor were used to protect vaginal walls. Total operative time was 315mins with 30min break in the middle. The estimated blood loss was 200ml and cystoscopy at the end of the procedure was reassuring. The specimen weighed 730gms.
Results
She made full recovery and discharged home after 48 hours. Follow up at 6 weeks indicted no complication and normal return to function and patient satisfaction. Histology confirmed benign fibroid.
Conclusion
Operative time cannot always be accurately predicted. It is better to have the resilience and skill to deal with unexpected intraoperative findings laparoscopically when possible.

FCVP30- Case report: An interesting case of complex pelvic pathology associated with subfertility – demonstration of investigative work up and operative management

Donna Ghosh[1*], Tom Aust[1]
[1]*Wirral University Teaching Hospital, Mersey, UK*

Case Report
A 38 year-old nulliparous woman presented to the fertility clinic with a history of recurrent first trimester miscarriage. She had a regular menstrual cycle associated with dysmenorrhoea and previously treated chlamydial infection but no other relevant medical or surgical history. Blood tests suggested normal ovulation, thrombophilia and endocrine screen was negative and both patient and partner karyotype was normal. Transvaginal ultrasound demonstrated a bulky uterus with a 5cm sub-serous fibroid, a right ovarian dermoid cyst and a possible left ovarian endometrioma. MRI further identified a bi-cornuate uterus and showed the 76mm sub-serosal fibroid arising from the posterior myometrium and projecting posteriorly to the left pelvic side-wall. The right kidney was seen within the pelvis, abutting the right ovary, which contained the dermoid cyst.
Management options were fully discussed and the patient counselled with regard to potential impact on fertility and future pregnancies if surgery was undertaken along with explanation of the risk of oophorectomy and hysterectomy. A CT IVU was performed pre-operatively to delineate the urinary tract in relation to the pelvic structures and help plan surgical approach.
Laparoscopy confirmed the pelvic imaging findings as well as rectovaginal endometriosis and bilateral endometriomas. Hysteroscopy identified a uterine septum. The patient underwent laparoscopic myomectomy, with extensive adhesiolysis, right ovarian cystectomy and hysteroscopic septoplasty.
Discussion
We present the full review of this case of subfertility along with relevant USS, MRI and CT imaging for discussion. The video presentation

contains demonstration of anatomical variation and pathology and the surgical techniques for management.

FCVP31- Stretching laparoscopic instruments to their limits, a case of of total laparoscopic hysterectomy of big fibroid uterus weighed>1000gm

Oudai Oudai[1*]
[1]*West Cumberland Hospital, Whitehaven, UK*

Introduction
There are case reports and series of laparoscopic hysterectomies with very large weights. There are not well defined limitations to laparoscopic approach. Intraoperative assessment is important to check mobility and access to the sidewall of the pelvis to allow progression with laparoscopic route.
Case
49y female with previous two vaginal deliveries presented to the surgical team with pyrexia, with acute abdominal pain and pyrexia with pelvis mass up to the umbilicus. She was also anaemic from menorrhagia for more than 6 months required antibiotics and blood transfusion and imaging indicated 14x12x13 uterine complex mass causing right hydronephrosis. She improved on conservative measures with Esmya and booked for hysterectomy with the intention of intraoperative assessment for laparoscopic hysterectomy.
Methods
Using 10mm scope with 30 degree angle at Palmers point it was possible to easily access the left side of the pelvis with bladder reflection. Mobilising the right side was difficult as the whole uterus was rotated and pressing on the sidewall but ultimately achieved. G2 Enseal articulating sealing device was particularly useful. Retrieval was done vaginally with the help of Alexis ring retractor to protect vaginal walls and vault was closed vaginally. Part of a laparoscopic grasper was missing intraoperatively.
Results
This was retrieved separately at a later procedure within 24h and ultimately patient made full recovery. The specimen weighed 1100gm and histology indicated necrotic infected fibroid.
Conclusion
Achieving laparoscopic hysterectomy is still the least traumatic route with the best enhanced recovery if safely achievable. However, the weight and mobility of the uterus can put significant strains on laparoscopic instruments

Poster Presentations

P1-A case of incarcerated and calcified GyneFix intra-uterine device successfully and safely removed endoscopically

Osama Eskandar[1*]
[1]*North Devon Hospital, Barnstaple, UK*

Introduction
A case of intrauterine incarcerated and impacted GyneFix IUCD is described. It was removed successfully hysteroscopically.
Case report
A 55 year old woman was referred to the Gynaecology outpatient clinic. Her GP had attempted to remove the Gynaefix however, it was found to be firmly fixed and traction on the strings caused considerable pain to the patient. The patient underwent hysteroscopy and D&C and removal of incarcerated GyneFix. The GyneFix was found calcified and incarcerated at the fundus of the uterus and was removed hysteroscopically.
Discussion
The GyneFix is a "frameless" IUCD, consists of six copper sleeves, each 5 mm long and 2.2 mm in diameter.(1) It is inserted by a needle through knot, at a depth of 1 cm, into the fundal myometrium. Due to its frameless design, flexibility, and minimal presence in the uterine cavity, the GyneFix is associated with few expulsions and dysmenorrhea than the IUCDs.
An incorrect technique may increase the risk of perforation.(2) The fact that the GyneFix was found calcified, suggests that it had been partially perforating the myometrium. However, the patient did not manage to conceive after the insertion of the coil. A frameless device anchored in the myometrium might erode through more easily than a framed device.(5)
Conclusion
Although it is rare, the possibility of incarceration, migration and late perforation highlights the importance of the routine regular post insertion check up. Incarcerated or partially perforating GyneFix can be safely removed hysteroscopically.

P2- A close shave in the management of rectovaginal endometriosis

Naomi Bullen[1*], Matthew Browning[1], Mark Coleman[1], Jonathan Frappell[1]
[1]*Derriford Hospital, Plymouth, Devon, UK*

Introduction: Endometriosis affects bowel in 3-37% of all cases. There is ongoing debate regarding the necessity of either segmental resection or full-thickness disc rectal excision. This study aimed to review the use of the laparoscopic rectal shave technique to assess outcomes and patient satisfaction.
Methods: A retrospective database was established for patients requiring operative management of rectovaginal endometriosis between 2009 and 2014. Both electronic records and case notes were reviewed and data was collected on pre-operative symptoms, surgical procedure, length of stay, complication and re-admission rates and post-operative symptom recurrence. In addition patients answered a telephone satisfaction questionnaire.
Results: 52 patients underwent surgery during the study period with 69.2% carried out by a combined colorectal and gynaecological surgical team. Average age was 33.9 (21-43). Out of 52 patients 90% underwent rectal wall shave, 8% had segmental resection and 2% hysterectomy in combination with rectal shave. Surgery was performed laparoscopically in 96% with a 0% conversion rate. Average length of stay was 1.5 days with a re-admission rate of 5.8%. Major complications occurred in 2% with no patients requiring re-operation within the early postoperative period. Overall 17.3% went on to have further surgery for their endometriosis although only 3.8% required bowel resection. Patient satisfaction levels were high with a high proportion of patients having symptom improvement post surgery.
Conclusion: The laparoscopic rectal shave is a successful technique for the management of rectovaginal endometriosis with low complication rates and high patient satisfaction. Outcomes are improved by using combined colorectal and gynaecological surgical teams.

P3- A Decade of Fibroid Morcellation at UCLH

Elisabeth Bean[1*], Thomas Holland[1], Alfred Cutner[1], Ertan Saridogan[1]
[1]*University College London Hospital, London, UK*

Introduction:
Following recent concerns regarding laparoscopic myomectomy, we investigated the outcomes of patients who had undergone laparoscopic myomectomy at UCLH.
Methods:
A retrospective review of all women who had laparoscopic myomectomy from January 2004 to June 2014 at UCLH.
Results:
250 patients underwent laparoscopic myomectomy. Average number and size of fibroids removed was 1.89 (*1-10*) and 71.2mm (*10-150mm*) respectively. Breach of the uterine cavity occurred in 9.6%. The morcellator was used in 240/250 of cases. Average blood loss was 215ml (*minimal-1500ml*) and 6 cases had blood loss >1000ml. Average post-operative

admission was two nights (*0-7*). Post-operative complications included one wound haematoma, one readmission due to urinary retention and one case of small bowel obstruction. No procedures were converted to laparotomy after attempting laparoscopic myomectomy.
One additional planned laparoscopic myomectomy was converted to laparotomy due to sustaining a bowel injury during initial entry (previous history of bowel surgery during endometriosis treatment). Another patient had a uterine mass inconsistent with a benign fibroid at laparoscopy. The procedure was terminated after biopsy, which later showed a malignant leiomyosarcoma. All morcellated fibroids had benign histology.
Discussion:
In this subject group, morcellation was successful and safe, with no cases of morcellation of malignant tissues.
Conclusion:
In experienced hands, laparoscopic myomectomy appears to be safe and effective. When a fibroid appears suspicious, a biopsy should be taken and histology awaited prior to proceeding. Patient selection should be dependent on co-morbidities, in addition to site, size, number and location of fibroids.

P4- A multi-disciplinary approach to diagnosis & management- Caecal endometriosis versus a Gastrointestinal Stromal Tumour (GIST)

Paul Simpson[1*], James Hernon[2], Edward Morris[1]
[1]*Department of Obstetrics & Gynaecology, Norfolk and Norwich University Hospital, Norwich, UK;* [2]*Department of Colorectal Surgery, Norfolk and Norwich University Hospital, Norwich, UK*

Endometriosis has an estimated prevalence of 2-10% but this increases to between 10-25% in those presenting with gynaecological symptoms. About 12% of confirmed endometriosis cases are associated with extrapelvic disease, involving structures such as the lower gastrointestinal tract- the most common site being the sigmoid, followed by the rectum, the ileocaecal valve and the appendix.
A 42 year old patient with known endometriosis was managing her symptoms of dysmenorrhoea and non-menstrual pelvic pain with a Gonadotrophin Releasing Hormone (GnRH) agonist and add-back hormone replacement in the form of Tibolone. This had been effective for a duration of three years but a decision was taken for definitive surgery and a laparoscopic bilateral salpingo-oophorectomy was booked.
Concurrently she was under investigation for chronic anaemia and low ferritin. A colonoscopy was undertaken, which demonstrated a 10-12mm smooth sub mucosal lesion arising from the opening of the appendix. The clinical impression was of a possible Gastrointestinal Stromal Tumour (GIST) or carcinoid. The patient was offered a choice between surveillance and surgical excision.
Histological examination of the tissues confirmed endometriosis within the ovarian tissue and the polyp arising from the appendix base. These findings suggest the 'exuberent pedunculate polyp of 0.9cm' may have developed as a result of endometriosis.
Changes in the appearance of the serosal and mucosal surfaces of the large bowel in response to deposits of endometriosis are unpredictable. Therefore, any suspicious gastro-intestinal lesion in patients with endometriosis requires a multi-disciplinary approach to investigation and management to ensure appropriate management.

P5- A new regional anaesthetic block technique for laparoscopic surgery providing enhanced recovery

Malcolm John Dickson[1*]
[1]*Rochdale Infirmary, Lancashire, UK*

Post operative pain control is an important element of promoting rapid recovery and hospital discharge following day case laparascopic surgery. Yet only 60% of patients are satisfied with their analgesia.
Ultrasound guided TAP blocks provides very effective pain relief following laparoscopy. And allow patients to be fit for hospital discharge 25% faster. However, the procedure is highly operator dependant, requires expensive equipment and takes time - So any benefit it offers is negated by getting through fewer patients on an operating list. These drawbacks may explain why its use has never become popular.
A novel technique is described where local anaesthetic agent is placed into the transversus abdominus plane (TAP). This is done under direct vision, when the laparoscope is being introduced with a direct cut down technique. Results of its use show that women having gynaecology laparoscopy as a day case are fit for transfer from theatre recovery to the discharge ward 24% faster (28 mins vs 37 mins. p value 0.03) It is planned to run a randomised trial
It is hoped that as pain is better controlled in the immediate post op phase, this effect will persist and provide more rapid hospital discharge and reduced analgesic demand
There are in excess of 250,000 laparoscopic procedures carried out per annum in the USA. Whilst not all of these are day cases, if this technique allowed patients to be fit for discharge even 20 minutes earlier, there are substantial financial benefits to be made.

P6- A pathway for the management of patients with PMB, once endometrial cancer is excluded

Zenat Nazzal[1*], Aarti Mohindra[2], Mary Connor[1]
[1]*Sheffield Teaching Hospitals NHS FT, Sheffield, UK;* [2]*University of Sheffield Medical School, Sheffield, UK*

There are clear management pathways for investigating women who present with postmenopausal bleeding (PMB). The first task is to exclude endometrial and other cancers. However, once this has been accomplished there are no pathways for subsequently managing women. For example, it is not clear at what endometrial thickness women should be offered hysteroscopic examination, and which endometrial polyps, once identified, should be removed. It can be expected that 10 - 20% of women presenting with PMB will have endometrial polyps; others will have recognisable atrophic vaginitis or, by a diagnosis of exclusion supported by features in their history, a resurgence of ovarian activity.
We performed a retrospective review of a cohort of patients who attended with PMB during the first six months of 2014. After excluding women with endometrial and other cancers we collected the following information: endometrial thickness found on (transvaginal) ultrasound scan; findings at hysteroscopy if performed, including the presence of endometrial polyp(s); the histology results of any removed polyps. We developed models to see how many women would be offered hysteroscopy for three given endometrial thicknesses: 5, 7 and 10 mm and for each of these thicknesses how many women would have polyps identified (or missed) and if any unsuspected malignancies were subsequently identified (or potentially missed). This information will help us define what endometrial thickness in our PMB patients should trigger subsequent hysteroscopic investigation, and so enable us to plan our outpatient hysteroscopy sessions and further develop our 'See & Treat' for endometrial polypectomy.

P7- A patient information leaflet for hysteroscopic examination: a proposed generic leaflet

Alex Sutcliffe[1*], Katharine Tylko[2], Elaine Falkner[2], Mary Connor[1]
[1]*Sheffield Teaching Hospital, NHS FT, Sheffield, UK;* [2]*Macmillan, CancerVOICE, London, UK*

There is good evidence that patients who receive written information about a procedure prior to attending a hospital appointment experience less anxiety. However, the content of the leaflet is important, as well as how the information is presented, and considerable effort is required when writing such leaflets to ensure that they are comprehensive and can be understood.

Our outpatient hysteroscopy leaflet was due for revision and a new version was produced. However, following last year's BSGE meeting it was apparent from a patient representative that our new leaflet might still not be adequate. For example, it was still insufficiently clear that patients have a choice between outpatient and inpatient examination and investigation, and some of details about the stages in the outpatient investigation were lacking clarity.
We therefore asked for comments from a group of patient representatives about another version of the leaflet and compared ours with those from other units to see if we could develop one that would be more patient-friendly and available to all units providing a hysteroscopy service. The new patient-friendly leaflet will be presented.

P8- A proposal to introduce Cognitive Apprenticeship in Gynaecological Endoscopy Training

Suruchi Pandey[1*]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK., Chertsey, UK*

Traditional teaching, especially in technical fields, used the apprenticeship model. Collins et al. in 1991 described the instructional teaching model of Cognitive Apprenticeship (CA), which is a situated teaching model where the expert is required not just to demonstrate the technical (or clinical) procedure, but also to vocalize their thinking, so that the students can learn from their thought process and the various points experience that informed the teacher's decision-making. Thus, CA can be an extremely effective means of training in Gynaecological endoscopy.

CA encompasses 6 key teaching methods:

1. Modelling: the expert shows the students how the task is done, while vocalizing their thought process
2. Coaching: the students perform the task under expert supervision
3. Scaffolding: the expert "fades" as per student needs
4. Articulation: the students articulate their thoughts during performing the task
5. Reflection: the students reflect on the tasks they performed and compare their performance to expert performance
6. Exploration: the students perform tasks independently

A study undertaken by Stalmejer et al has demonstrated that CA is not being used to its full potential in clinical medicine due to shortage of time available to clinical teachers, short placements of students, clinicians while modelling not describing why they were doing what they were doing, clinicians being unaware of the exact stage of student's learning and more emphasis on assessment than feedback. Also, reflection and exploration, though deemed as the 2 most useful components of cognitive apprenticeship, remained largely unused.

P9- A prospective cohort study to investigate the effectiveness of a simulation based structured stepwise approach to diagnostic laparoscopy in gynaecological trainees

Kirana Arambage[1*], Dilip Visvanathan[1]
[1]*John Radcliffe Hospital, Oxford, UK;* [2]*Whipps Cross University Hospital, London, UK*

Background: The outcome of the RCOG trainees' survey in 2010 has showed that laparoscopic surgery is one of the top areas of deficiency among gynaecological trainees in the UK. Thus, this 10 step structural stepwise training module was proposed to fulfil training needs in novice trainees in gynaecology.
Aim: To investigate the effectiveness of a simulation based structured stepwise training module on diagnostic laparoscopy in gynaecological trainees in The North East Thames region using box trainers.
Method: This prospective cohort study was proposed to investigate whether the simulation based structural stepwise training module (SSTM) would improve knowledge, skills and perception in gynaecological trainees in the North-East London denary. The impact of SSTM on the trainees were determined using pre and post test score differences between knowledge (MCQs, SEQ), skills (objective and subjective assessments) and perception (reaction evaluation form).
Results: The results demonstrated that the proposed SSTM significantly improved the knowledge and skills in novice gynaecology trainees from ST1-ST3 (p=0.000). The maximum % improvement observed in an individual for of MCQ SEQ, objective and subjective assessment were 45%, 50%, 60.5% and 57.5% respectively. Furthermore, a significant relationship between previous experience in laparoscopic surgery and pre-test scores for skills (p=0.03 for objective pre-test, p=.0.01 for subjective pre-tests) were observed in the trainee cohort. The % improvement of knowledge or skills after simulation were not related to the demographic factors (P>0.05).
Conclusion: Based on above results, this study concluded that proposed SSTM is an effective tool for laparoscopic simulation in novice trainees.

P10- A rare and unusual necrotising fasciitis with E.Coli as complication of laparoscopy

Ramawadhsingh Motolall[1*], Sean Watermeyer[1]
[1]*Royal Glamorgan Hospital, Llantrisant, UK*

Necrotising fasciitis is a rare and potentially fatal infection characterised by rapid and progressive involvement of the fascia and subcutaneous tissue. Early diagnosis and appropriate debridement of the infected tissues, with aggressive antibiotics therapy, remains the mainstay of the treatment. This is the case of a 22 year old lady who underwent a straight forward diagnostic laparoscopy for chronic pelvic pain, during which, no cause for her pain was identified. It was an uneventful procedure but this was shortly followed by an overwhelming necrotising fasciitis of the anterior abdominal wall from the suprapubic port insertion. A subsequent CSU culture indicated that she probably had an asymptomatic coliform UTI at that time. Intraoperatively, there was a 'through and through' injury to the bladder, presumably by the Veress needle insertion, which extravasated infected urine around the subcutaneous tissue of the anterior abdominal wall, bladder and peritoneal cavity. Her condition was extremely poor but she was managed appropriately by surgical debridement and antibiotics and thus escaped a catastrophic event.

P11- A rare case of acute non-puerperal uterine inversion managed by laparoscopically-assisted vaginal hysterectomy

Kinza Younas[1*], Ben Chisholm[1], Joshy Neelankavil[1], Euan Kevelighan[1]
[1]*Singleton Hospital, Swansea, UK*

Aim
To highlight a rare cause of severe abdominal pain and vaginal bleeding, and describe a laparoscopic approach to managing non-puerperal uterine inversion.
Case Report:
A 47-year-old nulliparous female presented to the emergency department with sudden onset, severe abdominal pain and vaginal bleeding. Her history included menorrhagia and 2cm fundal fibroid. She was pale and vomiting with severe pain despite 20mg of intravenous morphine. On examination, her abdomen was firm and her uterus impalpable. A round, broad-based mass lay within the introitus, acting as bung to profuse vaginal bleeding. An expelled Mirena coil lay posteriorly. An extruded fibroid polyp or uterine inversion was suspected.
In theatre, vaginal examination revealed almost complete inversion of the uterus with a fundal fibroid attached. On laparoscopic entry, the uterus was found to have formed a funnel into which the fundus, corpus,

fallopian tubes and round ligaments had retracted. After a successful laparoscopically-assisted vaginal hysterectomy, the patient was discharged well the following day.

Conclusion

Acute non-puerperal uterine inversion is rare with fewer than 150 cases reported until 2006. It should be suspected in women with sudden onset pain and bleeding, an impalpable uterus and a vaginal mass. We have found only one other report of non-puerperal uterine inversion managed by laparoscopically-assisted vaginal hysterectomy1. The procedure is complicated by the distortion of the pelvic anatomy resulting from uterine inversion, with the ureters being brought into close proximity of the ovarian and uterine vessels by traction on the vascular pedicles.

P12- A retrospective descriptive comparison study of transvaginal ultrasound scan findings with hysteroscopy and histology findings on postmenopausal women who underwent hysteroscopy for postmenopausal bleeding

Chellappah Gnanachandran[1,2*], Paul woollett[1], Peter Kimani[1], Kumaran Mylvaganam[1], Amit Patel[2], Feras Izzat[1]

[1]University hospital coventry and Warwickshire, Coventry, UK; [2]University hospital Bristol NHS foundation Trust, Bristol, UK

Objective:

To define an endometrial thickness, that helps to identify postmenopausal women with a high risk of having an endometrial polyp.

To bring clinical effectiveness in investigation for postmenopausal bleeding

Design: Retrospective descriptive study. Outcomes were compared with histology outcomes.

Population: postmenopausal women who underwent a transvaginal scan followed by hysteroscopy within 28 days of the scan over a two year period (390 cases)

Results: 143 (37%) women had ultrasound findings suggestive of an endometrial polyp and 140 (36%) had a thickened endometrium but no evidence of an endometrial polyp on USS. The remaining 107 cases (27%) had inconclusive USS findings, and an endometrial polyp could not be excluded.

When a scan was suggestive of an endometrial polyp in postmenopausal women, the positive predictive value of a polyp in histology was 82%.

When a scan was not suggestive of an endometrial polyp (140), 41% (57/ 140) had no polyps but 59% (83/140) had polyps on histology.

Mantel-Haenszel common odds ratio estimate shows that the statistical significance of the risk of having an endometrial polyp is higher when the endometrial thickness is 9mm and above compared to if the endometrial thickness is 8 mm or below.

Conclusion: Difference in detection rate of endometrial abnormality and polyps in patients with postmenopausal bleeding shown ineffectiveness in present clinical pathways.

For a protocol to decide the need of operative hysteroscopy or hysteroscopy under general anaesthesia, for postmenopausal women, an endometrial thickness of 8 mm cut off can be used.

P13- A story of a patient and her ovarian monsters

Gabriella Yongue[1*], Tariq Miskry[2]

[1]Luton and Dunstable University Hospital, Luton, UK; [2]St. Mary's Hospital, London, UK

Background: Mature ovarian teratomas account for 10-20% of all ovarian tumours and are most common in women aged 20-40 years. It is reported that 8-15% occur bilaterally and recur in just 4.2% of cases following surgical excision.

Case Presentation: We report a case of a 39 year old nulliparous female who has had six operations over the last 17 years to excise a total of 19 ovarian dermoid cysts that recurred bilaterally. Her first two initial ovarian cystectomies were performed by laparotomy whilst the last four procedures were laparoscopic. During all laparoscopic procedures, there was no spillage of cyst contents into the abdomen or pelvis and the dermoids were all removed using an endocatch bag. There were no residual cysts left behind at the end of each operation.

Discussion: There is little in the literature about why ovarian teratomas recur after surgical excision and what steps can be taken to prevent this. It has been suggested that recurrence of ovarian teratomas are more frequent following laparoscopy as opposed to laparotomy. However, this case demonstrates that dermoid cysts can recur after laparotomy and perhaps with the advantages of minimally invasive procedures, such as swifter post operative recovery, laparoscopy should prevail as the gold standard.

P14-Acceptability of Out-patient Hysteroscopy procedures

Sreebha Rajesh[1*], Rebecca Hardcastle[2], Chris Guyer[1]

[1]Queen Alexandra Hospital, Portsmouth, UK; [2]Royal Hampshire County Hospital, Winchester, UK

Aim: To assess patient tolerance to hysteroscopic procedures at the ambulatory Gynaecology clinic at Queen Alexandra Hospital, Portsmouth.

Materials and Methods: Data was collected prospectively over a two year period from January 2013 to December 2014. Primary outcome was pain score on a 10-point visual analogue score at the beginning, mid proceure, on completion and in recovery. We also looked at the additional analgesia requirements post procedure and complication rate. The data was analysed using SPSS.

Results: A total of 61 procedures were performed. Essure sterilisation (26.2%), Myosure polypectomy (23%) and Endometrial ablation procedures - Novasure (21.3%); Minitouch ablation (16.4%) and Hydrothermal ablation (13.1%). Median Visual analogue score mid procedure was highest for Novasure(7). (whisker plot attached). However, in recover the Median visual analogue score dropped down to 2 for all procedure except HTA with a VAS of 1. Only 4 (6.5%) patients required additional analgesia in recovery and 2 (3.2%) patients had a vasovagal episode.

Conclusion: Essure, Myosure and endometrial ablation procedures are well tolerated by patients in the Out patient settings with low procedure related complications. Novasure has the hightest mid procedure visual analogue score but in recovery all patients reported a low pain score.

P15- An audit of the adherence to the 2 week wait referral pathway for patients with Post-Menopausal Bleeding

Thirumagal Bavananthan[1,2*], Hannah Lowden[1], Pratibha Arya[1], Mamta Pathak[2]

[1]Worcestershire Acute Hospitals NHS Trust, Redditch, UK; [2]Worcestershire Acute Hospitals NHS Trust, Worcester, UK

Aim To assess referrals of patients with postmenopausal bleeding are compliant with National cancer care pathway guidelines and to improve compliance with standards, as well as improve clinical efficiency and patient experience.

Methodology Retrospective audit on patients identified from 2 week wait referrals made to the Ultrasound department between July 2013 and April 2014. 138 patients were identified and data obtained.

Results Women with endometrial thickness <4mm were appropriately discharged in 80% of the cases. Of the 96 patients seen in Gynae outpatient department 19% were not examined. The pipelle biopsy was successfully obtained in 61% with most common cause of failed procedure being cervical stenosis. Endometrial carcinoma was identified in 6

patients accounting for 10% of those who underwent hysteroscopy. The target of 14 days was met in 58% of patients.

Discussion Dedicated ultrasound supported PMB clinics will be useful as 80% of the cases were appropriately discharged reducing the work load to the GOPD.

One-stop PMB clinics where USS, examination and biopsy can all be performed in succession, improves patient experience and reduces waiting time. It is reasonable to suggest that biopsy should be attempted in all patients in GOPD unless the patient opts out. Women with cervical stenosis should be referred to OPH to have the option for the procedure under local anaesthesia. Patients unable to tolerate speculum examination could be referred to out-patient hysteroscopy for vaginoscopy to avoid general anaesthesia where possible.

Implementation of a guideline with a flowchart followed by re-audit would be valuable.

P16- An investigation into laparoscopic entry technique preference amongst UK Surgeons and Gynaecologists - Assessment of the best practice and future training

Raj (Prithwiraj) Saha[1*], Pratyasha Saha[2]
[1]*Heart of England NHS Foundation Trust, Birmingham, UK;* [2]*3rd Yr Medic, Cambridge University, Cambridge, UK*

Although laparoscopic surgery is now practiced commonly as a first choice over open surgery, its major complications have not changed in the last 25 years. At least 50% of its major complications happen during access, and therefore worryingly even before commencement of the intended procedure. We carried out a comprehensive survey of over 150 UK Surgeons and Gynaecologists with the aim of investigating influences in the practice of different entry techniques. This included training, attitude to change and willingness to address any training issues. Our results highlight interesting trends in practice. Perhaps this is the first study where Surgeons and Gynaecologists are questioned simultaneously. 78% of our respondents were Consultants, 70% of them practicing over 10 years and 40% over 20 years clinical situation, over 50% had not changed their practice from what they were trained in. justify the views of this study from established doctors. Most of the gynaecologists indicated a preference for closed entry surgery whilst majority of our 38 general surgical respondents preferred open entry technique. This variance was mostly due to the way these senior practitioners were trained. Although 85% believe that methods of entry should be changed according to the patient's Despite a heartening 94% were in agreement that different methods of training should be made available, our study also indicates that an opposition to change is present in laparoscopy and should be addressed. We suggest that the curriculum of training in both these surgical specialties should be broadened to include wider training opportunities.

P17- An unusual cause of chronic pelvic pain: Pseudomyxoma Peritonei

Gemma Nightingale[1*], Renee Behrens[1]
[1]*Royal Hampshire County Hospital, Winchester, UK*

A 35 year old, para two, presented to the gynaecology department with a two year history of increasing suprapubic pain. It intensified mid-cycle and five days prior to her period. She complained of significant menorrhagia, dysmenorrhoea, deep dyspareunia and dyschezia.

At laparoscopy copious amounts of mucoid exudate was seen occupying the uterovesical fossa and surrounding both ovaries. Her uterus, fallopian tubes and ovaries appeared normal and there was no evidence of endometriosis. An inflammatory exudate was seen along peritoneal surfaces of the anterior abdominal wall with obvious adhesions on both lobes of the liver. The appendix appeared normal. A sample of the mucoid fluid was sent for microscopy and culture and the patient was treated for presumed pelvic inflammatory disease.

The infection screen was negative and the fluid showed pus cells with no bacterial growth. Her symptoms did not improve and she was therefore referred to a national specialist centre with a possible diagnosis of pseudomyxoma peritonei. Further investigations including CT scan, colonoscopy and repeat laparoscopy, confirmed the diagnosis of a perforated mucinous appendiceal tumour. She underwent complete cytoreduction with pelvic clearance, appendicectomy, splenectomy, cholecystectomy and omentectomy. All peritoneal surfaces were stripped including the diaphragms and liver capsule followed by heated intraperitoneal chemotherapy (HIPEC).

Although pseudomyxoma peritonei is a rare condition affecting one person per million per year, any surgeon operating within the abdomen may occasionally encounter this borderline malignant, slowly progressing tumour which has poor long-term survival rates of 10-30% over 10 years if untreated.

P18- Application of the principles of higher education to laparoscopic training

Suruchi Pandey[1*]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK., Chertsey, UK*

As we enter the golden era of gynaecological endoscopy, training future generations to provide high quality patient centred care gains paramount importance. In my review, I discuss how the theories of learning in higher education can be applied to laparoscopic training.

Firstly, I discuss, the need to develop an outcome-based spiral curriculum (in accordance with Biggs) for gynaecological endoscopy (GE), which is constructively aligned with the teaching delivered and the assessments used with an aim to facilitate the development of a holistically trained laparoscopic surgeon.

I elaborate on my proposal under the following subheadings:

1. Intended learning outcomes including development of specialist knowledge, organizational skills, intellectual skills and personal attributes.
2. Learning in GE: to promote a deeper approach to learning – to ensure that the students Remember, Understand and Apply well (as per Anderson et al.'s update to Bloom's taxonomy) and also spend considerable time Analyzing, Evaluating and Creating.
3. Teaching in GE to include cognitive apprenticeship, integrated longitudinal clerkship, peer controlled teaching, collaborative exercises and self-reflection.
4. Assessment of learning outcomes: Based on the work of Rust, I propose a wide variety of assessments spread throughout the year with emphasis on formative assessment and timely constructive feedback.
5. Evaluation of training provided: Trainees be encouraged to provide confidential training feedback to the BSGE on the adequacy of the curriculum, training methods and the assessments

These measures would hopefully enhance higher order thinking with appreciation of the principles of caring and the human dimension.

P19- Are we counseling patients correctly prior to Endometrial Ablations?

Rebecca Hardcastle[1,2*], Chris Guyer[1]
[1]*University of Surrey, Guildford, UK;* [2]*Ethicon, London, UK*

Background:

Despite following NICE guidance[2007] promoting Mirena/Ablation for heavy periods we noted that our hysterectomy rates had not decreased.

Objectives:
To investigate further intervention rates post second generation endometrial ablation.
Design:
We undertook a retrospective study looking at patients undergoing endometrial ablation procedures and their satisfaction/further intervention rates
A surgical coding report was produced containing patients undergoing an ablation procedure between January 2008 and December 2012 (5 years)
All 682 patients were contact via mail and asked to complete a survey, 287 responded.
Results:
64% of patients were satisfied with the outcome of procedure (i.e. symptom relief)
of this group 12% still required further treatment.
Of ALL patients 39.5% required further treatments
52% of Patients who failed to be satisfied with ablation and had previously failed with a mirena coil went on to require further treatment, much higher than the 22% of the group previously successful treatment with a mirena .
It is interesting that in the previous failure with Mirena group 34% went on to have a hysterectomy compared to none of those previous successful with a mirena..
Discussion:
Ablations are now in widespread use and perhaps we are using it above its limitations - meaning a higher failure with more difficult cases
Patients who have previously failed to be successfully treated with a mirena coil are at a higher chance of having a failed ablation procedure
More specifically a higher risk of Hysterectomy
If these patients were told of their increased further intervention risks they choose an ablation?

P20- Asherman syndrome

Victoria Asfour[1*], Georgina Fraser[1], Saikat Banerjee[1]
[1]*St Peters hospital, London, UK*

The outcomes of Asherman's syndrome after surgical correction have a wide variation in reported outcomes in terms of menses, conception and complications in pregnancy. The challenges and possible variables affecting the outcomes of hysteroscopic adhesiolysis is discussed in the context of 2 cases that went on to achieve ongoing pregnancy. In the first case, pregnancy was achieved after 4 months. Pregnancy was complicated by abruption requiring emergency caesarean section at 34 weeks and severe haemorrhage.The second patient is 31 weeks in an ongoing pregnancy.
In both cases the endometrial cavity was obliterated by adhesions. Neither tubal ostia were reached. Hysteroscopic location the adhesiolysis was confirmed by concurrent trans-abdominal ultrasound guidance. First case: The cavity was essentially normalised though the endometrium appeared denuded. Second case: 3D ultrasound gave the appearance of a subseptated uterus reaching down to the cervix. At hysteroscopy the septum had the hallmark appearance of fibrotic scar. Both tubal ostia were identified through a narrowed passage from the cervix to each tubal ostium on each side. The adhesions between the two were divided using a twizzle via a 0 degree angulated 4mm versascope (Gynaecare). In both cases a copper IUS was placed for 6 weeks. Both patients received 28 days of ethyl-oestradiol.
We propose an audit tool for Asherman's syndrome that could be used by clinicians to audit their outcomes, and if endorsed by the society could provide a reflection of the UK experience of the incidence, severity, management and outcome of this condition.

P21- Atypical polypoid adenomyomas

Anita Nargund[1*], Anthony Griffiths[1]
[1]*UHW, Cardiff, Wales, Cardiff, UK*

Atypical polypoid adenomyomas (APA) are rare tumours of uterine corpus, usually occurs in premenopausal group, presents with abnormal uterine bleeding. APAs are classified as benign mixed epithelial and mesenchymal tumour, was first described by Mazur in 1981.
The histology features are very similar to well differentiated endometrial carcinoma, in some cases it is difficult to distinguish between APA and endometrial cancer.
Even though APA is categorised as a benign lesion, recurrence or residual primary lesion has been reported to occur in 30.1% of cases that were managed conservatively. Moreover, endometrial carcinoma is detected in 8.8% of APA cases.
There have been cases reported with coexisting carcinoma or cases, which have progressed to carcinoma. Thus follow up is crucial in management of these cases.
We present a case of atypical polypoid adenomyoma, which was diagnosed after hysteroscopy and polypectomy prior to IVF treatment.

P22- Audit of 23 women diagnosed with miscarriage who attended University Hospital South Manchester Early Pregnancy Unit over a 3 month period

Sandra Sasson[1*]
[1]*University Hospital South Manchester, Manchester, UK*

Approximately 20% of clinical pregnancies end in miscarriage. The aim of this audit was to compare current practice with National Guidelines. Women attended the Early Pregnancy Unit between October and December 2014. Their ages ranged between 22 to 41 years of age. Parity ranged between Para 0 to Para 5. 22%(5/23) had a previous miscarriage and 13%(3/23) had a previous termination. 42%(8/23) were referred by their General Practitioner and 32%(6/23) from the Accident and Emergency Department. 43%(10/23) presented with abdominal pain and vaginal bleeding, 39%(9/23) with vaginal bleeding alone. The majority of women required 2 ultrasound scans for their diagnosis. 57%(13/23) were diagnosed with missed miscarriage and 43%(10/23) with incomplete miscarriage. Bloods were taken in 100%(23/23) of cases. 4%(1/23) required emergency admission for heavy vaginal bleeding. In women who chose expectant management, 75%(6/8) were successful. 25%(2/8) proceeded to medical management with 100%(2/2) success rate. In women who chose medical management, 67%(4/6) were successful. 33%(2/6) proceeded to surgical management with 100%(2/2) success rate. Their length of admission ranged from 22 to 62 hours. In women who chose surgical management, 100%(6/6) were successful. Their length of admission ranged from 6 to 9 hours. All women had consent forms completed and Anti-D administered if appropriate. Patient choice is paramount and psychological sequelae common, thus patients should have access to support and counselling. Alternative methods are becoming more widely available which help reduce length of hospital admission and improve patient quality of life. These include outpatient medical management and manual vacuum aspiration.

P23- Bowel Herniation into Pre-peritoneal Space in a 5mm port site

James Dilley[1*], Jenny Lo[1]
[1]*West Sussex Healthcare Trust, Sussex, UK*

A 45 year old G3 P3 with BMI 25 underwent a total laparoscopic hysterectomy with bilateral salpingo-oophorectomy for fibroid uterus and menorrhagia.

The procedure used 4 non-dilating ports (10, 5,5,5mm), a 20 French Robinson drain was inserted into left lateral 5mm port at closure. Other port sites were closed with Prolene.
The following day, the patient complained of localized lower abdominal pain 4 hours after drain was removed and a mass developed gradually at the port site. A presumed diagnosis of port side bowel herniation was confirmed by CT imaging showed a small bowel hernia associated with bowel obstruction.
Laparoscopy revealed mid-small bowel incarcerated hernia within the pre peritoneal space which was reduced laparoscopically. The patient made an uneventful recovery.
This case highlights the rare but known risk of herniation through a 5mm port after removal of drain[1]. Current RCOG guidelines suggest all lateral ports of less than 10mm do not need closure. The musculature of the abdominal wall that underlies the fascia and the fascia elasticity will normal prevent herniation[2]
A systematic review has found that slowly absorbable and non-absorbable sutures reduce risk.[3]There is evidence to suggest that a non traumatic, bladeless trochars in reducing risk of hernia.[4]
Diabetes, smoking, excessive manipulation of trocar and lower quadrant ports due to absence of posterior sheath may increase the risk. [5]Elderly patients and thin patients also have a reduced strength and elasticity of fascia so may be more prone to hernia[2].

P24- Carboprost: A Useful Adjunct in the Laparoscopic Resection of a Cornual Ectopic Pregancy

Rebecca Mallick[1*], Tosin Ajala[1], Tony Kelly[1]
[1]*Royal Sussex County Hospital, Brighton, UK*

Introduction
Cornual ectopic pregnancies are a rare, but very challenging form of ectopic to manage. Laparoscopic resection can be a successful fertility preserving treatment option, but there is always a high risk of haemorrhage/hysterectomy. We present the first case of its kind where intramyometrial carboprost injection was used successfully, prior to laparoscopic resection, to help delineate the ectopic, improve visualisation, aid resection and reduce potential blood loss in a challenging case.
Case Report
A 38-year-old woman presented to the EPU at 6-weeks pregnant with abdominal discomfort. A right cornual ectopic was diagnosed on scan and subsequently managed surgically. In theatre, the findings were a diffuse enlargement of the cornua with no discrete ectopic visible making attempted resection technically difficult and potentially hazardous. 500mcg carboprost was injected laparoscopically directly into the myometrium surrounding the ectopic. This resulted in localised myometrial contractions, pushing the trophoblastic tissue closer to the uterine surface and demarcating it more clearly for resection. Monopolar diathermy was used to make an incision over the ectopic and suction used to remove the POC. The incision was sutured laparoscopically and blood loss was <50mls. She was discharged home the following day and completely discharged 2 weeks later with a negative βHCG.
Conclusion
Cornual ectopic pregnancies remain a challenging clinical dilemma. Laparoscopic resection is a safe treatment option and the use of intramyometrial carboprost injection prior to resection appears to improve visualisation and delineation of the ectopic pregnancy, resulting in a technically more straightforward resection with significantly reduced blood loss.

P25- Case Report - Laparoscopic management of a 20cm ovarian cyst in the third trimester of pregnancy using direct optical entry as an alternative approach

Donna Ghosh[1*], David Rowlands[1]
[1]*Wirral University Teaching Hospital, Mersey, UK*

Introduction
The prevalence of ovarian cysts in pregnancy is reported as between 1-2%. The decision to operate in pregnancy is difficult with views on optimal management and timing of surgery being controversial.
Case Report
A 20-year-old primiparous woman presented with a 16x11x20cm multi-septated right adnexal cyst, identified at first trimester ultrasound. She had a past medical history polycystic ovarian syndrome and a BMI of 44. Tumour markers were normal. Conservative management with regular monitoring was agreed.
Following an acute admission with abdominal pain the patient underwent ultrasound-guided aspiration of the cyst at 23/40. Cytology showed no malignant cells. The patient had recurrent admissions with acute pain from 27-30 weeks gestation, managed conservatively due to risk of mid-line laparotomy, preterm labour and prematurity. USS confirmed re-accumulation of the cyst. The aim was for caesarean delivery by 32-34 weeks with right ovarian cystectomy/oophorectomy. At 30+4 weeks-gestation the decision was made for surgery in view of non-resolving symptoms and suspicion of torsion. The patient underwent laparoscopy via direct optical entry to the right-side of the abdomen with planned insertion into the ovarian cyst capsule. The ovarian vessels were identified, demonstrating evidence of multiple torsion. The patient underwent right salpingo-oophorectomy. A small grid-iron incision was made to remove the enlarged complex cyst. Histology confirmed ischaemic necrosis.
Discussion
We present this case report with contemporary review of the literature. We describe our laparoscopic approach as a safe alternative in the management of large ovarian cysts in advanced pregnancy.
1. Zanetta et al. BJOG 2003; 110:578-583.

P26- Case Series of Different Management of Caesarean Section Scar Pregnancy

Radwan Faraj[1*], Gemma Govinden[1], Hannah Yeeles[1]
[1]*Rotherham Hospital, Rotherham, UK*

Case1:
1st case was in her third pregnancy diagnosed at 7 weeks gestation when she presented with vaginal bleeding. Gestational sac was seen implanted at CS scar away from uterine cavity.
She was counselled o have systemic and local MTX. Dose calculation at 1mg/m2 and also next day she was given transvaginal ultrasound guided using double lumen egg collection needle. Initially sac content was aspirated then 1 ml KCL was injected till cessation of fetal heart activity. Then 40mg MTX was injected into the sac.
HCG follow up showed gradual decline till negative in 6 weeks.
Case 2:
The second case was 33 years old previous 1 CS. In this case we gave her systemic MTX only at 1mg/m2. Her initial response was good with decline of HCG by 60% over one week but plateau in the second week at 600 IU and 55IU. The sac was getting bigger, more vascular on Doppler.
Decision was to proceed with surgery in view of the size of the mass and plateuing HCG. We decided on vaginal approach with ultrasound guided surgical/suction evacuation. The procedure was started with a Shirodkar suture that acted as a tamponade to control any operative bleeding. The procedure continued by suction evacuation under ultrasound guidance. She stayed inpatient and suture was removed in 48 hours. Her HCG decline was steeper and became negative in 3 weeks.
Case 3:
This patient was managed by Ultrasound guided suction evacuation without the use of Methotrexate. Her HCG decline was satisfactory.

P27- Comparing laparoscopic and open surgical management of all endometrial cancer patients in the North West Thames Gynaecological Cancer Network

Rebecca Pugh[1*], Alan Farthing[1]
[1]Queen Charlottes and Chelsea Hospital, London, UK

BACKGROUND: Endometrial cancer is the fourth most common malignancy in women in the UK. NICE guidelines state that there is sufficient data on safety and efficacy to support the use of laparascopic total hysterectomy and laparoscopic assisted vaginal hysterectomy for the treatment of endometrial cancer.
METHOD: A retrospective audit was compiled looking at all women treated for endometrial cancer in the North West Thames Gynaecological Cancer Network from 1st April 2012 to 30th September 2012.
RESULTS: 62 women were identified as having had surgical treatment for endometrial cancer. 76% of cases were managed in a tertiary cancer unit and 71% of cases were completed laparoscopically. Many of the patients were obese (average BMI 32) and 66% of cases had stage 1A disease. Patients having a laparoscopic procedure stayed in hospital for a significantly shorter time than those who had an open procedure. Two laparoscopic procedures were converted to an open operation but there were a greater number of post-operative complications in those having open surgery. 45% of patients did not require any adjunctive treatment.
CONCLUSION: Despite the growing problem of obesity, the majority of patients still present with early stage disease. Most patients were operated on by a laparoscopic approach. These patients had fewer complications rates and were able to be discharged more quickly. Confirming that laparoscopic procedures are a safe and affective way of treating endometrial cancer.

P28- Comparing MRI and laparoscopic results of patients in an endometriosis MDT

Danielle Nimmons[1], Lakshmi Sandu Aana[1*], Peter Byrne[1], Sonali Limdi[1], Gaity Ahmad[1]
[1]Pennine Acute Hospitals NHS Trust, Greater Manchester, UK

Background and Aim
MRI has been shown to be an accurate and a cost-effective tool for the preoperative staging of deeply infiltrating endometriosis (DIE). Pennine Acute Hospitals NHS Trust is a recognised endometriosis centre. An MDT was established in March 2014 to aid management of the condition. This MDT includes gynaecologists, radiologists, urologists, and colorectal surgeons. The severity of disease can be assessed on diagnostic laparoscopy. However MRI is a non-invasive tool that can help women in making a choice of different treatment options. The aim of our retrospective quality improvement project was to compare preoperative MRI findings and diagnostic laparoscopic results.
Methodology
Fourty-five patients were included in an endometriosis MDT between 07/04/14 and 03/01/15. Thirty-six patients who had DIE on MRI were included in the study. MRI findings, MDT results, histology results and laparoscopic findings were collected for these patients. These were tabulated and analysed using Microsoft Excel.
Results
Twenty-five out of the 36 patients had surgery, 6 chose medical treatment and 5 are awaiting surgery. Furthermore, due to the MDT a patient was identified as having diverticular disease and not endometriosis. Overall, approximately 80% of MRI findings correlated with laparoscopic findings. Specifically, this was 92% for bladder endometriosis, 76% for recto-vaginal, 64% for rectal, 80% each for left and right endometrioma; and 83% for bilateral endometriomas.
Discussion and Conclusions
MRI is beneficial for localising and mapping DIE. It helps plan MDT approaches to surgical management and helps women to make an informed choice.

P29- Complications and Length of Hospital Stay following Total Laparoscopic Hysterectomy: Support for Daycase Laparoscopic Hysterectomy

Islam Abdelrahman Ramadan[1*], Sophie Elaine Slater Bennett[1]
[1]West Wales General Hospital, Carmarthen, UK

Background:
Laparoscopic hysterectomy has proved a cost saving and viable alternative to a total abdominal hysterectomy or a vaginal hysterectomy. Day case total laparoscopic hysterectomies are already being performed and these could lead to even further cost reduction due to the decreased hospital stay. However this needs to be evaluated carefully to ensure that patient's risk of post operative complications are not increased. Here we look at a small cohort of women who have undergone a total laparoscopic hysterectomy as an inpatient to evaluate whether this supports day case hysterectomies.
Aim:
To evaluate the complications, length of hospital stay and patient satisfaction after total laparoscopic hysterectomy in a district general hospital in order to provide support to the possibility of outpatient (day case) laparoscopic hysterectomy
Methods:
A retrospective analysis of 64 women who had undergone a total laparoscopic hysterectomy in a district general hospital.
Results:
Of the 64 women booked for total laparoscopic hysterectomy, there were no major intra-operative or immediate post-operative complications. The average haemoglobin drop was 1.75 g/dL. The median hospital stay was 2.5 days (one of these being pre-admission) and the median period of recovery to normal activity was 3.5 weeks.
Conclusion:
The low rates of complications, minimal blood loss coupled with the already short hospital stays (the average length of stay post procedure was 1.5 days) suggest that total laparoscopic hysterectomy as a day case is a safe, viable and potentially cost saving procedure for a large number of patients.

P30- Correlation of MRI findings with findings at laparoscopic surgery

Ronnie Rahman[1], Emmanuel Ofuasia[1*]
[1]Croydon University Hospital, London, UK

Our study going back 5 years looks at how MRI reports have correlated with actual findings at laparoscopic surgery. We discuss when differences have occured, its impact on patient management.

P31- Documentation of Laparoscopic procedures. How good are we at completing operative notes and discharge summaries?

Dana Touqmatchi[1*], Jennifer Davies[1], Sonal Dave[1], Ian Chilcott[1], Nick Nicholas[1]
[1]Hillingdon Hospital, London, UK

Accurate and legible documentation are prerequisites for delivering high-quality care.
Almost 250,000 woman undergo Laparosopic surgery yearly. Articles on safety entry techniques and operating are established, therefore every effort should be made to include these techniques on operation notes. In addition to

this, most laparoscopic day cases are discharged back to GPs, and with NHS England stating that 33% of hand written and 26% of electronic discharge summaries fell short of NICEs minimum standards for clinical communication, it is vital to ensure discharge summaries are clear and accurate.
Given this, an audit was performed to determine how well we documented our operative procedures and how accurate our discharge summaries were.
A prospective audit was completed which include 50 sets of notes.
Results: 90% of primary operating surgeon completed notes. 50% of attempts of veress entry were documented. Palmers test documented in 10%. Starting intraabdominal pressure documented in 72%. Reduction of pressure after trocar insertion documented in 34% of cases. 86% of operation notes were clearly documented. Suture material used for skin documented in 94%. Of those discharged back to GP, 80% had clear discharge summaries.
Results showed areas that can be improved on in terms of documentation, and we have devised a sticker that can used on operating notes to help document key steps. Other recommendations include computerised documentation.
The discharge summary can be improved by ensuring a registrar or consultant reviews this, as this is usually left up to the most junior member of the team to complete.

P32- Does the addition of bilateral salpingectomy (BS) at laparoscopic hysterectomy increase morbidity?

Obi Ojukwu[1*], Alice Hurrell[1], Funlayo Odejinmi[1]
[1]*Whipps Cross University Hospital, London, UK*

Background: Recent evidence suggests the fallopian tubes may be the origin of certain serous ovarian cancers. Post-hysterectomy the fallopian tubes are redundant and can cause hydrosalpinx, pelvic pain and ectopic pregnancy. Increasingly more women are undergoing prophylactic BS and studies demonstrate that BS does not affect ovarian function. However, does it affect short-term morbidity?
Objective: To assess whether BS at total laparoscopic hysterectomy (TLH) or laparoscopic subtotal hysterectomy (LASH) influenced surgical time, blood loss or length of hospital stay.
Method: A retrospective case-control study. The cohort consisted of 306 consecutive single surgeon laparoscopic hysterectomies in a London hospital. BS or bilateral salpingo-oophorectomy (BSO) was used selectively. Results were analysed using the Student t-test and Mann-Whitney test. Significance was a p-value of <0.05.
Results: 57 out of 112 (51%) had a TLH, 21 (19%) had a TLH-BS and 34 (30%) had a TLH-BSO.
117 out of 194 (60%) had a LASH, 50 (26%) had a LASH-BS and 27 (14%) had a LASH-BSO.
LASH-BS was significantly associated with lower blood loss and shorter length of hospital stay compared to LASH alone or LASH-BSO. TLH-BS was significantly associated with lower blood loss compared to TLH alone or TLH-BSO, and significantly shorter surgical time compared to TLH-BSO.
Conclusion: We conclude that the addition of BS at laparoscopic hysterectomy is not associated with short-term morbidity. Lower blood loss may reflect the surgeon learning curve. This bolsters evidence that women should consider BS at laparoscopic hysterectomy and this information can be used during informed consent.

P33- Encephalitis associated with dermoid cyst - case report and review of literature

Monika Oktaba[1*], Lucy May[1], Andrew Baxter[1], David Paling[2], Basil Sharrack[2], Julia Palmer[1]
[1]*Sheffield Teaching Hospital; Department of Obstetrics and Gynaecology, Sheffield, UK;* [2]*Sheffield Teaching Hospital; Department of Neurology, Sheffield, UK*

Case presentation:
A 29 year-old office worker presented to hospital with history of headache, pyrexia, aggressive behaviour and hallucinations.
Her past history included depression treated with Sertraline, which was discontinued one week prior to admission.
Gradually she became obtunded with dystonic attacks and developed seizures.
Her condition deteriorated further requiring ICU care and intubation.
After several investigations she was diagnosed with autoimmune encephalitis.
CT pelvis showed 14 mm ovarian dermoid cyst.
Anti-NMDA encephalitis was suspected and decision was made for oophorectomy.
NMDA receptor antibody was negative in serum but positive in cerebrospinal fluid.
She has been making gradual clinical recovery.
Discussion:
The N-methyl-D-aspartate receptor (NMDA) is involved in normal physiological and pathological states in the brain.
Anti-NMDA encephalitis is characterized by memory deficits, seizures, confusion, and psychological disturbances in males and females of all ages. This type of encephalitis is often associated with ovarian teratoma in young women.
Treatment includes immunotherapy e.g. high-dose intravenous corticosteroids, immunoglobulins, plasma exchange, cyclophosphamide, azathioprine, mycophenolate mofetil, tacrolimus, methotrexate, and monoclonal antibodies (e.g., rituximab) in sequence or in combination. Some patients may recover to their normal state with supportive care alone. Most of the patients required further treatments such as tumor resection and immunotherapy. It has been proposed that the tumour should be removed when present.
Recovery may take 2 years or longer, and the patient may not always return to their former levels of motor function and cognition. Prognosis is better after tumor resection and immunotherapy.

P34- Endometrial ablation using radiofrequency in women with previous Caesarean Section: is this a safe procedure?

Gudrun C. Rieck[1*]
[1]*Betsi Cadwaladr Health Board, Bangor, Gwynedd, UK*

Introduction:
The device used radiofrequency for endometrial ablation (NovaSure®). The machine switches itself off when the impediance is too high.
Aim:
Does the device perform safely in cases with previous Caesarean Sections if the thickness of the scar is unknown?
Method:
Prospective safety audit between March 2011 and August 2014. Most women had an ultrasound but the caesarean section scar was only mentioned once. All women undergone a hysteroscopy before insertion of the device.
Results:
94 patients were listed for endometrial ablation. In eleven cases endometrial ablation was not attempted as there were unexpected clinical findings at the time of the diagnostic hysteroscopy.
83 cases undergone endometrial ablation.
In four cases (5%) endometrial ablation device was introduced but the procedure was abandoned by the machine: one case had large fibroid, one case cavity was probably too wide, one case a possible instrument failure. One case had a very thin lower segment as documented on pre-op scan (one case out of the 20 cases with previous caesarean section 5%).
79 patients had endometrial ablation performed. Out of those 19 cases had previous LSCS: 6 cases had one previous LSCS, 3 cases had two previous

LSCS, 8 cases had three previous LSCS, one case had four previous LSCS. One case had a history of uterine perforation.
There was no acute complication.

P35- Endometriosis care pathway and the role of an Endometriosis Specialist Nurse

Michelle Davies[1*], Suruchi Pandey[1], Saikat Banerjee[1], Shaheen Khazali[1]
[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK., Chertsey, UK*

The role of an Endometriosis specialist nurse (ESN) varies from centre to centre and within each centre, the role will evolve over time with experience. In this poster, we share our experience in setting up a post for a new ESN and the effects this has had on our service provision. We explore various duties and responsibilities of the ESN and also share our integrated endometriosis care pathway for the benefit of other developing centres.
Our ESN started in the year 2014. Her job was initially funded by the innovation fund and later her salary was paid by the income generated by her clinics.
Following duties are performed by our ESN:
Triage and co-ordinatation of care between teams and with GPs
Educating junior doctors and nurses
Collating and entering data into the BSGE database
Running patient support groups and follow-up clinics
Managing and updating the website, patient information leaflets and care pathways
Telephone follow-up
Providing direct telephone access for patients
Counselling and supporting patients throughout their treatment journey
Supporting ward nurses in caring for patients who had complex surgeries
Contributing to audit and research
The ESN has proven to be an invaluable asset to our centre

P36- Essure hysteroscopic sterilisation : Compliance with NICE guidance, our experience

Anita Nargund[1*], Caryl Thomas[1], Richard Penketh[1]
[1]*UHW, Cardiff, Cardiff, UK*

Essure hysteroscopic sterilisation is minimally invasive outpatient method of female sterilisation.
This method has many advantages including no incision, high successful bilateral placement rates, non-mandatory use of anesthesia, out patient procedure, Low level of pain, and low risk surgery. Therefore it is highly recommended than traditional laparoscopic sterilisation, which has more serious complications.
This type of sterilisation is particularly useful in patients with high BMI, with multiple previous abdominal surgeries.
The Essure system consists of two microinserts comprising a dynamic outer coil and an inner flexible coil, which are placed hysteroscopically into the fallopian tubes under direct vision.
Essure hysteroscopic sterilisation was performed in Outpatient procedure clinic, University hospital of Wales.
We present successful placement rates, pain scores during the procedure and outcomes at 3 month follow up.
It is a safe, quick, effective and irreversible method of sterilisation.

P37- A new regional anaesthetic block technique for laparoscopic surgery providing enhanced recovery

Malcolm John Dickson[1*]
[1]*Rochdale Infirmary, Lancashire, UK*

Post operative pain control is an important element of promoting rapid recovery and hospital discharge following day case laparascopic surgery. Yet only 60% of patients are satisfied with their analgesia.
Ultrasound guided TAP blocks provides very effective pain relief following laparoscopy. And allow patients to be fit for hospital discharge 25% faster. However, the procedure is highly operator dependant, requires expensive equipment and takes time - So any benefit it offers is negated by getting through fewer patients on an operating list. These drawbacks may explain why its use has never become popular.
A novel technique is described where local anaesthetic agent is placed into the transversus abdominus plane (TAP). This is done under direct vision, when the laparoscope is being introduced with a direct cut down technique. Results of its use show that women having gynaecology laparoscopy as a day case are fit for transfer from theatre recovery to the discharge ward 24% faster (28 mins vs 37 mins. p value 0.03) It is planned to run a randomised trial
It is hoped that as pain is better controlled in the immediate post op phase, this effect will persist and provide more rapid hospital discharge and reduced analgesic demand
There are in excess of 250,000 laparoscopic procedures carried out per annum in the USA. Whilst not all of these are day cases, if this technique allowed patients to be fit for discharge even 20 minutes earlier, there are substantial financial benefits to be made.

P38- Evaluation of the 2-week referral system for urgent investigation of postmenopausal bleeding in a District General Hospital setting Maria Boland MBBS BSc , Mark Broadbent BSc MFFP FRCOG

Maria Boland[1*], Mark Broadbent[1,2]
[1]*University College London, London, UK;* [2]*Barnet General Hospital, London, UK*

Aim: To investigate the incidence of endometrial carcinoma in patients screened according to the NHS 2-week target for postmenopausal bleeding.
Methods: Cross-sectional study undertaken at one NHS District General Hospital. Retrospective analysis of all patients presenting to our Gynaecology department under the 2-week referral pathway over one year. Hysteroscopy was offered to patients with endometrial thickness of greater than 5mm on TVUSS. Ultrasound findings, hysteroscopy findings, and histology results were correlated with clinical history to determine the incidence of endometrial carcinoma. Inclusion criterion was postmenopausal bleeding. Multips and primips were included. Women on hormonal treatment were excluded from the study.
Results: 107 referrals were received during this period. The mean age was 72 (range=50 – 86). 51 women (48%) underwent biopsy. Of these, 6 cases of endometrial cancer were detected, equating to 5.6% of all referrals.
Conclusions: The incidence of endometrial carcinoma in patients presenting with postmenopausal bleeding is 5.6%. Less than half of all referrals during this time met the criteria for hysteroscopy and biopsy. NICE guidelines (2012) infer that the pick up rate is expected to be 10%. These findings suggest that the two week wait results in over referral for invasive and costly investigations for women who do not have a diagnosis of endometrial carcinoma.

P39- Factors Affecting Surgeons' Decisions about the type of Hysterectomy they perform: A Qualitative Study

Sujata Gupta[1,2*]
[1]*University of Surrey, Surrey, UK;* [2]*LTHTR NHS trust, Preston, UK*

Summary- Majority of hysterectomies in the UK are performed through an abdominal approach. This is despite robust evidence in favour of vaginal and laparoscopic approach.This study aimed to find out what

factors influence decision regarding the mode of hysterectomy offered. Although this question has been addressed in various studies in the past, no UK study has explored this.

Aim -The main aim of this study was to explore the gynaecologists' views and factors that influence surgical decision making on the type of hysterectom offered.

Methodology-This is a qualitative study for which NHS consultants practising benign gynaecology in the northwest of England were interviewed. A total of 22 participants were interviewed.

Results-Key factors influencing surgical decision-making were surgeons' perceptions of an increase in clinical indications of hysterectomy for which an abdominal route was more appropriate. Abdominal route was also often preferred due to familiarity. They felt that there was a lack of training in vaginal and laparoscopic hysterectomy. Due to this, many surgeons expressed concerns about higher complications with minimal access routes in their hands. Low case load, time pressures and lack of organisational support were other influencing factors which favoured abdominal route.

Conclusions Most surgeons prefer abdominal hysterectomy as this is the route they feel most comfortable with. Lack of training and experience are the main contributing factors limiting the use of minimal access approach. Most surgeons want to develop their laparoscopic skills but similar desire is lacking towards vaginal hysterectomy.

P40- Findings of a quality improvement survey

Suruchi Pandey[1*], Alex Robinson[1], Michelle Davies[1], Saikat Banerjee[1], Shaheen Khazali[1]

[1]*Centre for Endometriosis and Minimally Invasive Gynaecology-CEMIG, Ashford & St. Peter's Hospital, Chertsey, UK., Chertsey, UK*

In order to improve the quality of care we offer to our patients, we are undertaking a telephonic survey of patients who had treatment of endometriosis in our unit. It is an on-going survey and the results are interesting.

The information below is based on the data available as of now. On an average the patients suffered for 5 years with pelvic pain before seeing the GP. 6 patients saw the GP more than 10 times before being referred to a specialist clinic. The gap between GP referral and appointment with the endometriosis team ranged from 2weeks-4months. The satisfaction scores with initial consultation, explanation of the disease condition, ability to directly access an endometriosis specialist nurse were high being 9.3, 8.7 and 9.5 out of 10 respectively. The patients felt that their views were respected with the average score being 9.1. However, not all were happy with the explanation of surgery preoperatively with the average score being 8.3. The likelihood of recommending the unit to a friend or family was 9.8.

The key areas where the patients wished to see improvement were – GP education regarding endometriosis symptoms, waiting times for surgery, operation cancellation, inability to see consultant post op due to split site working, inability to see consultant on follow up and being discharged too soon after surgery.

Even when there were complications, patients commented on the care being excellent emphasizing the importance of candour, good communication and teamwork.

P41- Grading quality of preoperative endometrial biopsy in endometrial cancer

Rasiah Bharathan[1*], Lim Teo[1], Mena Farag[1], Desmond Barton[1], Paul Bulmer[1]

[1]*St. Georges Hospital, London, UK*

INTRODUCTION

When women present with postmenopausal bleeding, following an ultrasound or hysteroscopic assessment, an endometrial biopsy may be performed. This biopsy informs the clinician regarding the histological subtype and the grade of differentiation of the cancer. Accurate preoperative grading is important in determining the place of care, counselling the patient about the extent of recommended surgery and forewarning the patient regarding potential adjuvant chemotherapy. This retrospective study examines the grading quality of preoperative biopsy.

METHOD

Patients who were diagnosed with endometrial cancer between 2008 and 2013 were identified through the histopathology database. The sensitivity, specificity, positive and negative predictive values and grading accuracy of preoperative biopsy was determined using final histology.

RESULTS

During the six year period, 158 women had surgical treatment for endometrial cancer. 46 patients were excluded because of lack of complete dataset or because of sarcomatous diagnosis. Complete set of data was available for 112 women. The sensitivity of preoperative grade 1, 2 and 3 are 61.5%, 61.5% and 81%, respectively. The specificity of grade 1, 2 and 3 diagnoses are 91.7%, 73.3% and 84%, respectively. PPV of G1, G2 and G3 are 80%, 41% and 71.4%, respectively. NPV of G1, G2 and G3 are 81.5%, 86.3% and 90%, respectively. The overall accuracy of G1, G2 and G3 are 80.4%, 70.5% and 83%.

CONCLUSION

Preoperative biopsy grading performance is moderate in quality. Randomised comparative trials of sampling techniques are required to define performance benchmarks. Accurate preoperative grading has potential implications for patient counselling, logistics, outcomes and resourcing.

P42- Gynaecological day case surgery: A snap shot view to improve cost-effectiveness

Lakshmi Sandu-Aana[1*], Rizwana Yakub[1], Uzma Ahmed[1], Sarah Carter[1], Gaity Ahmad[1]

[1]*Royal Oldham Hospital, Oldham, Manchester, UK*

BACKGROUND: The current NICE recommendation is for 75% of elective surgery to eventually be performed as day-case. Effective day-case surgery minimises hospitalisation whilst optimising efficient patient care and cost-effectiveness.

AIMS: Our service set-up for gynaecological surgery comprises of two sites specifically for day-case and one for inpatient procedures. Day-case procedures are routinely being done in the operating list at the inpatient site and this significantly increases 'bed pressure'. The aim of doing this study is to assess the efficiency of operative care and timely discharge of the day case procedures being done at the inpatient site.

SUBJECTS AND METHODS: We prospectively reviewed 100 day case patients at the inpatient site. We obtained the following information (1) Type of the procedure (2) Intra-operative/Post-operative complications (3) Reason for delayed discharge

RESULTS: 20% of the patients were not discharged on the same day. 9 % of patients had medical reasons for delayed discharge. The common reasons for extended stay included delayed medical review, consultant choice and afternoon surgery. There were no intra-operative complications. 6% of patients developed postoperative complications such as pyrexia, high drain output and anaesthetic complications.

CONCLUSION: Our study has highlighted the lack of local protocol and areas for improvement to avoid unnecessary inpatient stay at hospital. We aim to develop robust local protocols which stipulate; a clear pathway and co-ordinated nurse-led discharge under clinical leadership to make this service cost-effective in the current NHS climate.

P43- Gynaecological review in the ED. A trainees perspective

James Rowland[1*], Andrew Watson[1,2]
[1]*NW Deanery, Manchester, UK;* [2]*Tameside General Hospital, Gtr. Manchester, UK*

Introduction:
To evaluate the views of O&G trainees regarding gynaecological assessment in emergency departments in the Northwest England.
Methods:
An anonymous internet survey tool was used to gather data.
Results:
Of the 156 surveys, sixty two (62) responses were received. Fifty-six (55.7%) of those responded. Most trainees are working in units with greater than 4500 deliveries per annum. Fifteen percent (14.7%) and eighty six (86.3%) of the examinations are done by ED doctors and O&G doctors respectively. Ninety one percent (90.9%) have an agreed protocol for referral to emergency pregnancy assessment unit (EPAU). Fifty five (54.8%) stated that there is no direct access to emergency gynaecology outpatients at their hospitals and only forty eight percent (47.9%) have a referral protocol in A&E to the clinic. Forty four percent (43.9%) confirmed a lack of rooms with adequate patient privacy. Approximately a third (32.5%) reported that speculum examinations are not performed due to lack of privacy in A&E. Two thirds (66.7%) of the trainees state that they have encountered delayed referrals by A&E staff. Nearly half (46.3%) of the trainees state that patients aren't reviewed by medical staff in A&E before being transferred to a O&G ward setting.
Conclusion:
The majority of gynaecological examinations in emergency departments appear to be being performed by gynaecological staff. There appears to be strong evidence of suboptimal conditions for reviewing gynaecological patients. Clear protocols can improve patient satisfaction and reduce the burden on already overstretched departments

P44- Harmonic instrumentation significantly reduces the mental load compared with diathermy during simulated laparoscopic salpingectomy: A Randomised Cross-over Trial

Rasiah Bharathan[1*], Elias Kovoor[1]
[1]*Maidstone and Tunbridge Wells Hospitals NHS Trust, Maidstone, UK*

Introduction
Laparoscopic salpingectomy (LS) is an essential gynaecological procedure, which may be performed for tubal ectopic pregnancy or ovarian cancer risk reduction. Harmonic devices are purported to offer superior ergonomics. This study examines the impact of instrumentation on surgeons' mental load.
Method
Nine participants were stratified at recruitment to this cross-over randomisd comparative study, using computer generated allocation. Participants were first trained to proficiency in salpingectomy. All the performances were completed on LAP Mentor virtual reality simulator which captured the dexterity metrics and simultaneously recorded videos of the procedures. Each participant performed a salpingectomy using harmonic and diathermy (bipolar/monopolar) techniques during separate sessions. During the salpingectomy participants were required to perform simultaneously, a validated visuo-cognitive secondary task. After the procedures, participants completed two validated questionnaires - NASA-TLX and subjective mental effort questionnaire (SMEQ).
Results
In all six dimensions of NASA-TLX, harmonics resulted in significant improvement in workload measures [mental demand (P=0.02), physical demand (P=0.008), temporal demand (P=0.004), performance (P=0.007), effort (P=0.02) and frustration (P=0.003). SMEQ measure also reflected significant reduction in mental load associated with harmonic instrument (P=0.037).
The visuo-cognitive secondary task measures of mental load, revealed a significant reduction associated with harmonics: the overall detection rate (P=0.0004) and correct detection rates (P=0.0004) were significant.
Pearson correlation coefficient between mental load (NASA-TLX) and SMEQ was 0.74 for diathermy group and 0.76 for the harmonics group, thus demonstrating a moderately strong concurrent validity of outcome measures. This fortifies our findings.
Conclusion
Harmonic instrument significantly reduces mental load during simulated laparoscopic salpingectomy.

P45- Homemade Simulation Training - Does It Work?

Emily Gelson[1*], Helen Bolton[1], Ashish Pradhan[1]
[1]*Hinchingbrroke Hospital, Huntington, UK*

Aims/Objectives: To determine if a low cost task-based simulation training course, using household and DIY items, can improve laparoscopic surgical skills
Background: The surgical competencies required to perform laparoscopic surgery are challenging and require a period of intensive learning. The validity and benefits of simulation in gynaecological surgical training are being increasingly recognized. Indeed, the recent RCOG document 'Becoming Tomorrow's Specialist' recommends simulation training not only for trainees but also for continuous professional development throughout a career as a gynaecologist. Despite this, the availability of simulation training is patchy and costly.
Methods: Using household and DIY items twenty tasks were devised for individual use in a laparoscopic 'box trainer'. Sixteen gynaecology trainees spent six hours practicing these tasks. Each trainee was timed at completing four tasks before and after practice. Eight laparoscopically naive medical students were also timed at these four tasks.
Results: Compared to the medical students, the trainees did not perform the four test tasks significantly faster at baseline. However, after practice, the trainees showed a significant improvement in the median time taken to complete three of the four test tasks. On direct questioning all the trainees felt their laparoscopic skills had improved significantly as a result of practice.
Discussion: Time needed to complete a set task is a marker of hand-eye co-ordination and dexterity, an essential part of laparoscopic surgery. The overall improvement in the trainee's timings suggests practice improved these modalities.
Conclusions: A low cost, task-based simulation-training course can improve laparoscopic skills for gynaecology trainees.

P46- How do we best prepare our patients for their outpatient services appointment?

Fani Gkrozou[1*], Eve Gaughan[1], Richard Pyper[1], Bronwyn Middleton[2], Natasha Waters[1]
[1]*Southlands Hospital, West Sussex, UK;* [2]*St Richard's Hospital, West Sussex, UK*

Introduction: This study aims to assess the value of our patient information leaflet with regard to improving their out-patient hysteroscopy experience.
Material and Methods: We analyzed data from a questionnaire given to 74 women after outpatient hysteroscopy.

Results: 84% of women had received our information leaflet prior to their appointment. Of these women who received the leaflet in advance, 20% reported a pain score of 5 or less whereas only 8% of women who did not receive the leaflet reported pain scores of 5 or less.
Also, only 12% of those who had received our leaflet reported pain scores of 8 or more but 25% of the women who did not receive a leaflet reported such scores.
Only 14% of women took oral analgesia before the procedure.
85% of women were satisfied with the information leaflet they received before having hysteroscopy .
73% of women rated their experience as 10 (scale 0 - 10).100% were satisfied with the staff.
Women with no analgesia gave higher pain scores compared to those who took oral analgesic pre- procedure. There was no significant difference in pain scores between women taking only oral analgesics compared with those who had local analgesia.
Discussion: This study highlights the importance of a high quality patient information leaflet as reflected by the lower pain scores reported by the women who received information before the procedure compared to those who did not.

P47- How does histopathology correlate with the indication for hysterectomy?

Ilias Nikolopoulos[1*], Pinky Khatri[1]
[1]*James Cook University Hospital, Middlesbrough, UK*

Hysterectomy is one of the most common gynaecological procedures and provides a definitive cure for many benign diseases, but is sometimes associated with significant morbidity. Alternatively there are effective conservative treatment options available for such conditions.
Objective:
To correlate the indication for hysterectomy with the histopathology.
Design:
In this retrospective study, 189 hysterectomies performed at a University teaching hospital during a one year period were analyzed. The indications for surgery and the histopathological findings were correlated.
Results:
The majority of the procedures were done laparoscopically (54.5%) and vaginal hysterectomy was done in 35.5% of the cases.
The commonest indication for hysterectomy was genital prolapse in 58 cases (31%) followed by menstrual disorders in 56 cases (30%) and chronic pelvic pain/endometriosis in 51 cases (27%).
Sixty one percent of specimens from cases of chronic pelvic pain/endometriosis showed abnormal histology. Analyzing menstrual disorders further, when the indication for hysterectomy was DUB (without endometrial ablation), abnormal histology was found in 30% cases only. Whereas if it was done for post ablation pain or bleeding, abnormal histology was found in 73% of the cases.
Discussion:
Histopathology from cases of chronic pelvic pain/endometriosis and post ablative problems showed abnormal pathology in majority of the cases vindicating the need for surgery. However, when the indication was DUB (without offering ablation), only one third cases showed abnormal pathology. Hence it is prudent for each unit to conduct regular audit of hysterectomies correlating indication and diagnosis, in order to justify the need for major operative intervention.

P48- Hysteroscopic sterilisation : An advancing alternative to laparoscopic surgery?

D Tannous[1*], SJ Bonner[1], JE Davies[1]
[1]*Blackpool Victoria Hospital, Blackpool, UK*

Hysteroscopic sterilisation by tubal cannulation and placement of intrafallopian implants has been approved by NICE since 2009 and current literature suggests it is an effective, safe and well tolerated alternative to laparoscopic sterilisation.
The Essure insert system was introduced early, locally within the North West and as there are no clear national auditable standards we performed a retrospective case series of patients who have had hysteroscopic sterilisation performed from 2011 to 2015 (n= 60).
Through excel, data has been analysed to confirm adherence to NICE and FSRH guidance looking at consent, patient information, safety and procedure performance. Particular interest locally is to improve patient compliance with follow up in confirming bilateral tubal occlusion and to look at complications to maintain high procedural success rates and patient satisfaction, comparable to national data. Local guidance will be produced from this data to provide auditable standards.
At present we are yet to fully analyse the data however provisional results show hysteroscopic sterilisation is a safe, cost effective and well tolerated procedure with high rates of patient satisfaction. To date, both short and longer term complications are low and suggest to be less prevalent than with laparoscopic procedures.

P49- Indication and Outcome of Repeat Hysteroscopy Under General Anaesthesia Following Outpatient Hysteroscopy

James McLaren[1*], Abi Oladipo[1], AshleyMehmi[1]
[1]*Croydon University Hospital, London, UK*

Aim: To determine the indication and outcome of patients referred for hysteroscopy under general anaesthesia following outpatient hysteroscopy at Croydon University Hospital.
Method: Retrospective audit of hysteroscopies performed under general anaesthesia following outpatient hysteroscopy over a 3 month period at Croydon University Hospital. Indication for referral, correlation of operative findings, and outcome were reviewed
Results: 89 hysteroscopies performed under general anaesthesia, 30 (22 for PMB, abnormal PVB >45yo, 1 HMB <45yo, 5 incidental findings of thickened endometrium) of these were referred following an outpatient hysteroscopy appointment. 14 were referred due to unsuccessful hysteroscopy - 12 unable to enter (2 of these remained unsuccessful under GA), 2 withdrew consent. 9 were referred due to diagnosis of polyps during the outpatient procedure - 2 of these had an attempt at polypectomy (1 unable to tolerate, 1 unsuccessful 21mm), 7 had no attempt at polypectomy (1 had no polyp on repeat hysteroscopy, 3 had polyps <15mm, 3 had polyps 20-40mm). 6 were referred due to insufficient sample (4 of these had normal hysteroscopic findings). 1 was referred due to persistent PMB (atrophic endometrium and insufficient sample during outpatient procedure). 14/16 successful diagnostic outpatient hysteroscopies correlated with the intraoperative findings under GA. No malignancies were diagnosed in this small group of repeat hysteroscopies.
Conclusion: Insufficient histological sample with normal outpatient hysteroscopy should not be an indication for repeat hysteroscopy under GA. Review of local skill and training in outpatient hysteroscopy and resection.

P50- Intergration of fast track and outpatient hysteroscopy services. Questions and answers to help to create a successful outpatient hysteroscopy service

Fani Gkrozou[1*], Eve Gaughan[1], Richard Pyper[1], Bronwyn Middleton[1], Natasha Waters[1]
[1]*WSHT, Worthing, UK*

Introduction:The leading role of hysteroscopy in the investigation of endometrial pathology is well known. With the development of new

technology and the expanding role of outpatient hysteroscopy it is very important to understand the benefits and safety of outpatient hysteroscopy. We combined outpatient hysteroscopy service and Fast Track service to investigate postmenopausal bleeding and created a pathway to streamline the patient's journey. **Material and Methods**: We performed a 6 month retrospective study of 165 post menopausal women, referred from Fast Track to outpatient Hysteroscopy Clinic and seen within two weeks of referral. The indications were post menopausal bleeding and increased focal endometrial thickness (> 5 mm), suspicion of polyp on ultrasound, inability to obtain sample in Fast Track clinic and non-diagnostic sample. Rigid or flexi hysteroscopes were used. All women had endometrial biopsy. **Results**:Most of patients were able to be seen and complete investigation and treatment in One Stop Outpatient Hysteroscopy clinic. 10% declined outpatient hysteroscopy or had referral cancelled due to finding of endometrial carcinoma on Fast Track biopsy. Hysteroscopy in postmenopausal group revealed 29% normal endometrium, 44.24% endometrial polyps, 7.27% fibroids, 0.6% with endometrial cancer. No perforations were seen. 15% of patients needed cervical dilatation. **Discussion**:It is very important to take these results, showing a high rate of abnormal findings, into consideration and to create a pathway that can achieve better integration of these two services to investigate symptoms in women with high risk of cancer.

P51- Is Endometriosis a risk factor for ectopic pregnancy?

Michael Magro[1*], Alexandra Saetta[2], Funnmilayo Odejinmi[1]
[1]*Whipps Cross University Hospital, Barts Health NHS Trust, London, UK;* [2]*QueenMary University of London - Barts and the London School of Medicine and Dentistry, London, UK*

IntroductionThere are multiple risk factors for ectopic pregnancy. Tubal pathology, caused by previous surgery, pelvic infections or endometriosis[1,2] is known to be a strong risk factor. Endometriosis alone may double the risk[3], however some authors have disputed this[4-7]. We sought to investigate if endometriosis truly is a risk factor for ectopic pregnancy.
Methods Retrospective review of 698 patients with an ectopic pregnancy who underwent laparoscopic surgery between 2004-2014 at a multiethnic district general hospital in London. The presence or absence of endometriosis was identified and known risk factors compared between the 2 groups.
Results 36 (5.2%) had endometriosis and 662 (94.8%) had no evidence of endometriosis. 34 (94%) had grade 1-2 disease, 2 (6%) stage 3 disease and 0 stage 4 disease. Mean age was 31 in both groups. No significant difference between tubal or extra-tubal ectopic location (p0.33) was identified. Significantly, 75% with endometriosis were nulliparous and 0% had a previous ectopic pregnancy, whilst it was 46% and 11% respectively in those without endometriosis (p0.0008 and p0.03). 11% with endometriosis conceived using Artificial Reproduction Techniques (ART) and 4% without endometriosis (p0.04).
Discussion We believe that endometriosis per se is not a risk factor for ectopic pregnancy, at any site, as the prevalence in those with ectopic pregnancy over the last 10 years is equivalent to that of the general population; 2-10%[8,9]. However, those with endometriosis had a statistically higher chance of being nulliparous and needing ART, which does independently increase the odds of ectopic pregnancy.

P52- Is robotic sacrocolpopexy really necessary?

Heleni Mastoroudes[1*], Alexander Slack[1]
[1]*Maidstone and Tunbridge Wells NHS Trust, Kent, UK*

Objective: The use of robotic surgery is increasing rapidly. Proponents for robotic sacrocolpopexy(RS) advocate similar operating times, outcomes and a faster learning curve compared to laparoscopic sacrocolpopexy(LS). This is at the expense of high setup and procedural costs.
The aim of our study was to compare our unit's LS operating times to the published RS operating time data and the impact that this would have on a busy unit if RS were to become the 'standard'.
Method: We used an electronic theatre management system to identify LS performed between July 2011-March 2015 by a single,subspecialist urogynaecologist in a busy DGH. Demographic data included age,BMI,and parity. Perioperative data included,operating time,estimated blood loss,complications and length of stay.Six week follow-up looking at prolapse grade was used to calculate success rate.
Results: Seventy-three patients were identified, 21 were excluded due to concomitant procedures. Of the 53 patients that were included, the average age was 57.9 years, parity 2.3 and BMI 19-36. The mean operating time was 89.5 minutes compared to 230.5 minutes for RS. The average blood loss was less than 100 millilitres. There were no documented complications and the length of stay was 1 night. Both subjective and objective outcomes were good.
Conclusions: Most reports claim an equivalence between standard laparoscopic surgery and robotics in all but cost. In a unit with a high volume of LS, operating times are significantly shorter by a factor of almost 3. Combined with increased costs this would make RS entirely unfeasable in standard practice.

P53- Laparoscopic ability is an invaluable skill in unexpected cases

Lewis Nancarrow[1*], Tarun Ghosh[1]
[1]*Nobles Hospital, Isle of Man, Isle of Man*

A 39 year old was referred to Gynaecology clinic with recurrent dyspareunia and vaginal discharge 1 year after abdominal hysterectomy for menorrhagia.
Speculum examination showed granulation tissue in the vault and the plan was for removal of granulation tissue under anaesthesia.
On attempting to remove the tissue, further tissue prolapsed and it was thought to be fallopian tube in origin. This diagnosis was queried initially and the patient had been consented for laparoscopy. Having not seen a case like this before it was decided a laparoscopic approach would give us the conclusive diagnosis and on investigation it was a fallopian tube prolapse (FTP). Having the ability to perform laparoscopically allowed for quick definitive diagnosis and the patient had a laparoscopic bilateral salpingectomy and subsequent repair of vault defect vaginally under laparoscopic vision.
Systematic review by Ouldamer et al. found 51 cases of FTP following hysterectomy. This review looked at multiple management options, which ranged from silver nitrate application, resection of prolapsed tissue and then laparoscopic management. This review determined that laparoscopy was the most effective management with these cases with reduced recurrence and better symptom relief.
Having laparoscopic abilities at your disposal is invaluable in unexpected situations such as these and can greatly benefit patient management. The presentation of vault granuloma following hysterectomy needs to be reviewed as a potential FTP until proven otherwise. This is a rare complication following hysterectomy that can lead to potentially serious complications (peritonitis) and is a risk for clinicians to be aware of.

P54- Laparoscopic Bowel Injuries: The importance of early recognition and treatment

Alexa Lilley[1*], Jonathan Chamberlain[1]
[1]*City Hospitals Sunderland, Sunderland, UK*

Introduction
Complex gynaecological procedures are increasingly performed laparoscopically exploiting the benefits of shorter hospital stay and rapid recovery. Complications may be more subtle and not recognised

immediately with delayed treatment contributing to serious morbidity and mortality. The incidence of intestinal injuries is 0.06%-0.5% for diagnostic laparoscopy rising to 0.3%-0.5% for operative laparoscopy[1], relating mainly to entry complications, electro-surgery and port-site herniation.

Case Series

Patient outcomes following eight cases of bowel injury relating to laparoscopic adhesiolysis, excision of endometriosis, oophorectomy and hysterectomy are discussed. 37.5% of injuries were detected intra-operatively and better outcomes followed with no need for bowel resection, blood transfusion, additional surgery or readmission, with an average length-of-stay in this group of 2 days (range 1-3). Bowel injuries not recognised immediately had their diagnosis delayed by an average of 4.8 days (range 1-12) and 100% required stoma formation with an average of 1.8 additional surgeries (range 1-3) and 3.4 hospital readmissions (range 1-7). These patients stayed an additional 34.5 days in hospital (range 11-90). Intervention was delayed further if the patient was discharged before the injury was diagnosed (6.3 days) compared to those diagnosed postoperatively during the primary admission (2.5 days). Early warning scores and biochemical markers were not discriminatory, but symptoms of abdominal pain, anorexia and reluctance to mobilise were common features.

Summary

These cases highlight the significance of failing to promptly recognise bowel injury or post-operative deterioration. Gynaecologists should be vigilant through thorough patient selection, careful operative technique, assessments of bowel integrity and post-operative management.

P55- Laparoscopic Entry Technique – review

Monika Oktaba[1*], Andrew Baxter[1]
[1]*Sheffield Teaching Hospital, Department of Obstetrics and Gynaecology, Sheffield, UK*

Techniques used in laparoscopic entry depend on experience and preference of surgeon and complexity of operations undertaken.

At least 50% of laparoscopic complications occur prior to commencement of intended procedures.

Studies suggest that 30-50% of bowel injuries and 13-50% of vascular injuries are undiagnosed at the time of surgery.

To minimize entry-related injuries several techniques, instruments, and approaches have been introduced.

Closed entry – Veress needle

Most popular between gynaecologist.

Pre-peritoneal insufflation: 2.7%, 15%, 44.4% and 100% of cases at one, two, three, and more than three attempts respectively.

Umbilical infection: 1%.

Bowel injury: 0.04% - 0.2%

Vascular injury: 0.03% - 0.2%

Carbon dioxide embolism: 0.001%

Open entry – Hasson technique

Prevention of gas embolism and pre-peritoneal insufflation.

Longest laparoscopic entry, gas leak.

Umbilical infection: 0.4%.

Bowel injury rate: 0.05% - 0.5%,

Vascular injury: 0% - 0.005%

Direct trocar entry

Avoidance of failed pneumoperitoneum, pre-peritoneal insufflation, carbon dioxide embolism.

Fastest abdominal entry.

More than three attempts to enter the abdomen in 2.7%.

Failed technique: 0.4% - 1.4%

Bowel injury: 0.05% - 0.1%

Vascular injuries: 0%

Perforation of omentum: 4.8%

Optical trocars (Visula entry system)

Permits visually guided trocar entry without insufflation and easy recognition of injuries at the time of entry.

Quicker than Veress and Hassan

Bowel injury: 0% - 2%

Mesenteric injury: 0.1%

Vascular injury: 0.04%

Complication rate related to laparoscopic entry remained the same during the last 25 years. There is no clear evidence as to the optimal form of laparoscopic entry in the low-risk patients.

P56- Laparoscopic entry techniques - 2014 Cochrane Review update

David Gent[1*], Daniel Henderson[1], Kevin Phillips[2], Andrew Watson[3], Gaity Ahmad[1]
[1]*Pennine Acute NHS Trust, Manchester, UK;* [2]*Castle Hill Hospital, Cottingham, UK;* [3]*Tameside Hospital NHS Foundation Trust, Ashton-Under-Lyne, UK*

Background

Complications from laparoscopy are often related to the initial entry into the abdomen and range from life-threatening (e.g. visceral injury) to minor (e.g. extraperitoneal insufflation). There is no clear consensus as to the optimal method of entry. We present the second update to the 2008 Cochrane review on laparoscopic entry techniques**.**

Methods

The review used the search strategy developed by the Cochrane Menstrual Disorders and Subfertility Group. MEDLINE, EMBASE, CENTRAL and PsycINFO were searched up-to August 2014.

Results

46 randomised controlled trials with 7,844 individuals that underwent 13 different laparoscopic entry techniques were included. No advantage was identified of using any technique to prevent major vascular or visceral complications. Significant reduction in the incidence of failed entry, extraperitoneal insufflation, and omental injury for open-entry compared to a Veress Needle technique; and in vascular injury in the direct-trocar group compared to the Veress needle entry group were identified.

A reduction in trocar site bleeding was associated with radially expanding access system (STEP) trocar entry compared to standard trocar entry; and an advantage of not lifting the abdominal wall before Veress Needle insertion for failed entry.

Conclusions

There is a significant reduction in failed entry, but no difference in the incidence of visceral or vascular injury for open versus closed-entry techniques. Low rate of complications are associated with laparoscopic entry however small participants numbers within these studies may account for lack of significant difference in associated major complications between entry techniques.

P57- Laparoscopic Hysterectomy for Endometrial Cancer - Surgical Outcomes in a District General Hospital Cancer Unit

Helen Bolton[1*], Hema Nosib[1], Tarang Majmudar[1], Hisham Abdel-Rahmen[1]
[1]*Hinchingbrooke Hospital, Huntingdon, UK*

Background: Endometrial cancer is the most common gynaecological malignancy in the UK. Most women present with early stage disease when surgery alone may be adequate to achieve cure. NICE Guidance [IPG356] states that the current evidence on the safety and efficacy of laparoscopic hysterectomy for endometrial cancer is adequate to support its use. Laparoscopic hysterectomy is the offered route of choice offered in our Gynaecology Cancer Unit.

Aim: To investigate peri-operative outcomes for women undergoing surgery for endometrial cancer.

Methods: Retrospective case review audit comprising 2 years of patients undergoing hysterectomy for endometrial cancer within our unit.

Results: We identified 34 cases. Of these 31 (91%) had a laparoscopic procedure. Two open procedures were carried out due to pre-existing co-morbidities, and there was one conversion due to the presence of dense adhesions. There were no intra-operative complications. 32 had an estimated blood loss of less than 500ml (94%) and none required transfusion. There were no major post-operative complications. Six women experienced minor complications (18%) with just one requiring readmission. Our average hospital stay for laparoscopic hysterectomy was 1.5 days.
Conclusions: This audit has confirmed that we safely offer a laparoscopic approach to women requiring hysterectomy for early endometrial cancer within our Unit. Our rates of complications are comparable to reported rates in the literature despite our unit achieving one of the highest laparoscopic surgery rates in our region.

P58- Laparoscopic Hysterectomy for endometrial cancer: Analysis of eighty-seven cases from a cancer unit, Greater London

Rowena Sharma[1*], Panos Sarhanis[1]
[1]*Northwick Park Hospital, London, UK*

Background.
Endometrial cancer is the most common gynaecology cancer in the UK and often presents with postmenopausal bleeding. Stage 1 disease is treated surgically by total hysterectomy with bilateral salpingo-oophorectomy (BSO). Previously an open approach was mainstay, however use of total laparoscopic hysterectomy (TLH) with BSO is now supported as a surgical modality for endometrial cancer.
Materials and Methods.
Women proposed for surgery following multidisciplinary review for suspected gynaecology malignancy were included. All cases for TLH and BSO were included between 21/11/2011 and 27/02/2015. Information regarding patient age, surgery, length of stay, histology and complications was recorded onto an excel database. Information was analysed and compared to current NICE guidance.
Results.
Eighty-seven cases were included, the median age of the patient was 58yrs. Five cases underwent TLH only and the remaining underwent TLH and BSO. Results of histology demonstrated 39.4% were benign, 4.6% simple hyperplasia, 10.8% complex hyperplasia, 8.04% complex hyperplasia with atypia, 34.5% endometrial carcinoma, 2.3% metastatic endometrial carcinoma, 4.6% CIN, 1.15% GCIN, 1.15% borderline ovarian tumour 1C and 2.3% Lynch syndrome. The complication rate was 10.3%. The conversion rate to TAH was 3.45% (n=3). One case resulted in a laparotomy due to bowel perforation, there was one wound infection and four bladder injuries (4.59%). The median length of stay was 2 nights.
Conclusions.
Analysis of the cases performed in our unit is comparable to those reported in the literature supporting this method. As illustrated in this study it offers a number of advantages compared with open approaches.

P59- Laparoscopic hysterectomy training for endometrial cancer surgery: Validation of a virtual reality simulator

Rasiah Bharathan[1*], James McLaren[1], Thomas Ind[1]
[1]*Department of Obstetrics and Gynaecology, St.Georges Hospital, London, UK*

Introduction
Laparoscopy is the preferred route for hysterectomy. Surgical errors are more prevalent during the learning curve. High quality evidence supports simulation based training over standard training. Prior to adopting a simulator, the device must be tested for face and content validity. We present the first study of a hysterectomy simulator.

Methods
Twelve consultant and five subspecialty trainees who are certified to perform TLH for endometrial cancer, were recruited. The Simbionix virtual reality simulator offers a hysterectomy module and suturing tasks. The participants performed TLH + BSO and vault closure before completing the assessment. Participants rated the ten features of face validity and the seven features of content validity using a ten point Likert scale: a score of one equals minimum and ten indicates maximum. Qualitative feedback was captured.
Results
The face validity assessment scores were high, with median scores ranging 7-9: instruments' appearance (9), instrument manoeuvring (8), instrument functionality (8), tissue appearance (8), response to manipulation (7), depth perception (7), hand-eye coordination training (8), bimanual coordination training (8), value as a training device (8) ergonomics (7). The scores for content validation are uterine manipulation (9), IP ligament (8), bladder dissection (7), UA (8), colpotomy (7), identification of ureter (7) and vault closure (4).
Discussion
The study demonstrates face and content validity of the VR-LH simulator. The vault closure task on this VR simulator was not highly rated. We believe this simulator will enhance the learning of LH. The next steps would be assessments of construct validity and learning curves.

P60- Laparoscopic hysterectomy: Experience of a British district general hospital

Osama Eskandar[1*], Ibrahim Erfan[1]
[1]*North Devon Hospital, Barnstaple - Devon, UK*

Introduction Hysterectomy is the most frequently performed major gynaecological operation. In the UK the rate of hysterectomy is 42/100,000 population, with higher-rates in the United States (143/100,000) and 236/100,000 in Germany. It is interesting to note that while the trends showed a decline from the 1990's to early 2000, from 2002 till date the rates show a plateau.
Method This is a retrospective study of hysterectomies performed in North Devon District Hospital (NDDH) in UK over a period of one year (2014). Types of hysterectomies were analysed in light of the NICE guidelines standards November 2010 – interventional procedure guidance 356 and the Hospital Episode Statistics (HES).
Results 165 hysterectomies were performed during the period of the study. The rate of vaginal, abdominal and laparoscopic hysterectomy at the NDDH was 58.7%, 7.8%, 28.5%, compared to 35%, 66% and 3% of the national rate respectively.
The mean length of hospital stay after laparoscopic hysterectomy was 2 days compared to 4.6 days for abdominal hysterectomy. The mean operative time was 120 minutes compared to the national reported time in NICE guidelines of 111 – 168 min.
The rate of conversion was 5.4% compared to the reported 7% in the NIIC guidelines. The rate of intra-operative and post-operative complications was 2.8% and 5.6% compared to national rates of 10% and 17% respectively with a re-admission rate of 1.8%.
Conclusion This retrospective study reflects the current practice regarding one of the commonest gynaecological operations in one of the district hospital in the UK.

P61- Laparoscopic Location of sentinel lymph node in cervical carcinoma and factors affecting bilateral detection

Milica Perovic[1*], Rekha Wuntakai[1], Andreas Papadopoulos[1], Stephen Attard Montalto[1], Samar Geris[1], Luma Saad[1], Omer Devaja[1]
[1]*West Kent Cancer Centre, Maidstone and Tunbridge Wells NHS Trust, Maidstone, Kent, UK*

Retrospectively analysed data for the laparoscopic detection of the sentinel lymph nodes (SLN's) in early cervical cancer patients: FIGO Stage 1a1 (with LVSI) - 1b1. We looked at the anatomical location of the SLN and factors associated with unilateral detection, utilising intraoperative gamma probe and blue dye. All patients were consecutively treated between January 2005 and January 2015 at the Wet Kent Gynaecological Oncology Centre, Maidstone Hospital, Maidstone, UK.
The aim of the study was to document locality of SLNs in cervical carcinoma and examine factors affecting unilateral detection during laparoscopic surgical procedures.
A total of 84 women were investigate with a combined intra-operative approach. The 70% (N=59) of the patients presented in FIGO Ib1, 17% (N= 14) in Ia2 and 13%(N=11) in Ia1 stadium. In total 24 patients (28.6%) had lymph vascular space invasion (LVSI).
The most common SLN location was the external iliac region in 38.5% of the patients, 23.8% was in the internal iliac, with a percentage being identified in less common sites (obturator area, common iliac, para-aortic and pre-sacral areas).
SLN were detected unilaterally in 15 patients (17.9%), bilaterally in 66 patients (78.6%) and no sentinel node was found in 3 patients (3.5%).
Of all factors analysed only older age and higher BMI were associated with unilateral detection of the SLN.

P62- Laparoscopic management of a rudimentary horn pregnancy using EnSeal

Eve Rawlinson[1*], Swapna Ramasubramanian[1], Zul Anjum[1], Olubusola Amu[1]
[1]*Royal Oldham Hospital, Manchester, UK*

Background
Rudimentary horn pregnancy is a rare form of ectopic pregnancy with an incidence ranging from 1:76,000 to 1:140,000[1]. It carries a high risk of rupture, in the late first or mid-second trimester, with potentially life threatening intra-abdominal haemorrhage. Laparoscopic management is reported in only 9 cases. This is the first case of laparoscopic management using EnSeal.
Case
A 31 year old, G3P1, presented at 11 weeks gestation with lower abdominal pain. Ultrasound suggested an ectopic pregnancy within a non- communicating horn of the uterus. It was confirmed by MRI. The rudimentary horn and products of conception were removed laparoscopically using EnSeal. The patient recovered well and was discharged next day.
The rudimentary horn was dissected and ligated with EnSeal, it was cut into slices with EnSeal, allowing removal through the laparoscopic port.
Discussion
We present successful management of an 11 week gestation rudimentary horn pregnancy. EnSeal enabled bloodless dissection of the vascular pedicle and small sample size enabling easy laparoscopic retrieval. It further widens the scope of EnSeal in laparoscopic surgery.
Reference
1. [1] Nahum GG (2002) Rudimentary uterine horn pregnancy: the 20th-century worldwide experience of 588 cases. J Reprod Med 47:151-163

P63- Laparoscopic management of giant ovarian masses: case report of a 44-litre cyst

Victoria White[1*], Viviene Lee[1], Summi Abdul[1]
[1]*Royal Derby Hospital, Derby, UK*

The RCOG/BSGE guidance and Cochrane review state that laparoscopic management of ovarian cysts is preferable due to reduction in adverse intra-operative events, pain and length of hospital stay. However, there is insufficient evidence to guide our management of large or 'giant' ovarian cysts.

We aim to demonstrate that laparoscopic management of giant ovarian cysts is possible and may indeed be preferable in carefully pre-selected patients.
We report the case of a 44 year old lady whose CT scan revealed a massive pelvic mass 43x40cm, most likely ovarian in origin. Insufflation of the abdomen was not possible prior to deflation of the cyst and so 44 litres of serous fluid were drained through the umbilical port, after the trocar was inserted directly into the cyst. Once the sac was deflated it was removed through the lateral port after being bought to the surface in an endobag. The cyst wall was then extracted with Kocher's forceps. Histology confirmed a large benign paraovarian serous cyst.
Spillage of potentially malignant cyst contents should be avoided but according to the RCOG/BSGE, in pre-menopausal women, the incidence of ovarian cysts being malignant is only 1 in 1000. Therefore, after individual risk stratification and frank discussion with the patient laparoscopic cystectomy may be the most appropriate management. Management of giant ovarian cysts laparoscopically remains a controversial issue, however we demonstrate that with careful patient selection, avoidance of the pit-falls of open surgery is possible, even in the largest of cysts.

P64- Laparoscopic management of post myomectomy sequelae

Suku George[1*], Anita Thomas[2], Richard Hale[1]
[1]*Stockport NHS Foundation Trust, Stockport, UK;* [2]*St Helens & Knowsley NHS Trust, Whiston, UK*

A 37 year old Gravida 2 Para 0 lady was seen 10 months following a difficult myomectomy which was converted to a laparotomy from planned laproscopy. Presenting symptoms were ongoing discomfort and pain in the suprapubic region with an ultrasound showing a calcified 4 cm fibroid.
An MRI scan excluded a myoma but raised the possibility of a resolving collection in the myoma bed. With the histology of the fibroid while benign had features of a mitotically active myoma, a recurrence with degeneration was also suspected.
Follow up ultrasound scans failed to show resolution and a repeat laparoscopy was undertaken.
At laparoscopy a firm mass of 3 cm in the right lateral aspect of the lower uterus underneath the bladder reflection was noted which corresponded to the area of tenderness and imaging. Once the bladder was reflected below this are a 1.5 cm defect was apparent in the anterior myometrial wall with a well defined cavity containing a dark brown fibrous degenerated material. The cavity was evacuated and a thorough lavage carried out followed by a two layered closure with intracorporeal sutures.
Histological examination confirmed the material to be consistent with documented appearance for oxidized cellulose. On reviewing the original operative notes it was apparent for haemostasis , multiple sheets of surgical were used to pack the myoma bed and extra peritonised thus delaying reabsorption.
Post operative ultrasound 8 weeks late showed normal uterine contour with complete resolution of symptoms.

P65- Laparoscopic management of ruptured ectopic pregnancy with major intra-abdominal haemorrhage (Over 1.5 L): Case series and demonstration of operative technique

Mohamed Shahin[1], Sobha Ramakoti[1*]
[1]*Royal Stoke University Hospital, Stoke on Trent, UK*

Ruptured ectopic pregnancy with significant intra-abdominal haemorrhage is an indication for emergency surgical management. The choice between laparotomy and laparoscopy have been always subject to debate, with more growing reports supporting the feasibility and safety of laparoscopic treatment in such cases.
Traditionally, laparotomy was the preferred choice for those cases, especially with haemodynamically unstable patients, due to perception that

laparotomy is quicker and easier to achieve haemostasis in such cases. Factors affecting choice included: surgeon's, anaesthetist and theatre team laparoscopic experience, availability of laparoscopic instruments, patient observations and estimated intra-abdominal loss.
Our work represents case series of three cases of acutely ruptured ectopic pregnancies, with massive intra-abdominal haemorrhage (Range= 1500 - 3500 ml). All the 3 cases were managed laparoscopically, with haemostasis achieved within 5 minutes of the start of the procedure.
The technique demonstrated could not have been achieved with a knowledgeable supportive theatre team, involving anaesthetists, nursing staff and surgeons. The readily available laparoscopic instrumentation facilitated quick procedure and prompt haemostasis.
The technique included: Rapid perioperative resuscitation, +/- bedside US scan. No uterine instrumentation, 10 mm Zero degree scope, 10 mm supra-pubic and 5 mm lateral ports. Quick and efficient suction of blood using a powerful 10 mm suction system. Haemostasis quickly achieved using Endoloop or Ligasure. Non-use of irrigation. Alternating patient position between trendleberg and ante-trendleberg.
In conclusion: Laparoscopic surgery is safe and effective for managing ruptured ectopic pregnancy with significant bleeding. The volume of intra-abdominal bleed should not be a limiting factor in experienced hands.

P66- Laparoscopic Myomectomy - A ten year learning curve

Ravneet Sirha[1,2*], Reeba Oliver[2,1], Nilesh Agarwal[1,2], Funlayo Odejinmi[2,1]
[1]*Northwick Park Hospital, London, UK;* [2]*Whipps Cross University Hospital, London, UK*

Aim: Laparoscopic myomectomy (LM) is an organ preserving technique not only lending itself to enhanced recovery pathways, it has been described as the gold standard for certain fibroids and cases.
To review LM over a ten year learning curve, including peri-operative outcomes and resolution of symptoms/patients satisfaction.
Methods: Retrospective cohort study of 217 consecutive single surgeon LM performed at a London teaching hospital over a ten year period. A comparison was made between those cases performed in the first half of the data collection (first 5 years) with those performed in the second half.
Results: The demographics of the two cohorts of patients did not differ significantly. On clinical examination the size of fibroids selected were significantly larger in the second half of the study(13.26 vs 14.73, p=0.0089). There was no significant difference in the duration of surgery, number of fibroids removed and estimated blood loss over the ten years. There was an increase in the number of drains left in situ(0.44 vs 0.70, p<0.0001) and a significant decline in the number of complications (0.09 vs 0.02 p= 0.0156). In patient stay was reduced over time (2.40 vs 1.67, p<0.0001) whilst the resolution of patients symptoms improved (0.99 vs0.99, p=0.0014).
Conclusions: Our data shows that surgical performance of laparoscopic myomectomy improves over time and this is evidenced by a decline in complications and length of hospital stay. It also shows as surgical experience is gained the complexity of case selection increases, as does patient experience of symptoms resolution.

P67- Laparoscopic myomectomy - *an appropriate option for peri-menopausal women*?

Ravneet Sirha[1,2*] , Reeba Oliver[2,1], Nilesh Agarwal[1,2], Funlayo Odejinmi[2,1]
[1]*Northwick Park Hospital, London, UK;* [2]*Whipps Cross University Hospital, London, UK*

Aims: The decision to undertake a surgical approach in the management of uterine fibroids in peri-menopausal women can be controversial.
To compare peri-operative outcomes and symptom resolution of laparoscopic myomectomy(LM) in peri-menopausal and non menopausal women.
Methods: Retrospective cohort study of 217 LM performed between 2005 and 2013 for the management of uterine fibroids.
Results: The estimated blood loss(233.3 vs 305.1, p=0.2382) and drop in haemoglobin(1.13 vs 1.76, p=0.4512) was lower in the peri-menopausal group compared to non menopausal women, although this did not reach clinical significance. There was no difference in the use of surgical drains or length of hospital stay in both groups. The resolution of symptoms was comparable between patient cohorts, however the post operative patient satisfaction was greater in the non menopausal compared with the peri-menopausal women(0.91 vs 0.50, p=0.0128).
Conclusions: These findings suggest the surgical technique of LM is similar in both cohorts of women and not complicated by age. There was a slight reduction in estimated blood loss and drop in haemoglobin in the peri-menopausal group which could reflect the reduction in vascularity of fibroids at this age. Interestingly, whilst there was no difference in the resolution of symptoms between the groups, patient satisfaction was significantly less in the peri-menopausal women leading us to question whether surgical management is the most appropriate choice of management for them. This warrants further investigation into surgical recovery in peri-menopausal women and patient satisfaction with alternative forms of fibroid management to optimise treatment in this group of women.

P68- Laparoscopic port site Incision Hernia: A case report and review of literature

Meera Adishesh[1*], Tim Kundodyiwa[1]
[1]*Whiston Hospital, St Helens and Knowsley NHS Trust, Prescot, Merseyside, UK*

Introduction:
Port site hernia following laparoscopic surgery is less common compared to incisional hernia following open surgery. It is important to increase awareness among laparoscopic surgeons of the possibility of intrafascial incisional hernia as clinical findings may be subtle and early CT diagnosis is necessary for timely surgical intervention.
Case Report:
We report a case of port site hernia in a 67 yr. old woman who underwent laparoscopic bilateral salpingo-oophorectomy for a 16 cm right ovarian cyst with low risk of malignancy. Our practice is to close the fascial layer for any port sites more than or equal to 10 mm laparoscopically under direct view. She was readmitted with nausea, vomiting on post operative day 4. Conservative management was unsuccessful hence an urgent CT scan was performed, which showed small bowel obstruction due to herniation at the left port site (extended for specimen retrieval). She underwent laparoscopic release of port site hernia and the peritoneal defect was closed with GraNee's needle under direct vision.
Discussion:
Trocar/ port diameter and access technique can affect the rate of hernia formation. Port site hernias appear to be related to large diameter ports used for specimen extraction, older age group , high BMI, increased operative times and excess tissue manipulation leading to fascial weakening. However, in spite of primary fascial closure of ports, hernia is still reported.
We recommend meticulous closure of fascia and peritoneum for port sites above 7 mm, with GraNee's needle or laparoscopic suture passer closure system under direct vision.

P69- Laparoscopic subtotal hysterectomy with power morcellation. A single centre experience

Dimitrios Miligkos[1*], Ioannis Douridas[1], Adam Moors[1], Aderemi Kola-Adejumo[1], Sameer Umranikar[1]
[1]*Southampton University Hospital NHS Foundation Trust, Princess Anne Hospital, Southampton, UK*

Introduction:
Recent reports about the safety of power morcellation during laparoscopic subtotal hysterectomy (LSTH) have raised concerns because of the risk of intra-abdominal spread of unsuspected uterine malignancy. The AAGL and BSGE have released good practice guidelines for clinicians undertaking these procedures.
The aim of our study was to evaluate our practice of risk assessment, preoperative investigations and unexpected histology results in women undergoing LSTH.
Methods:
This was a retrospective single centre study from a tertiary university hospital. Consecutive patients that underwent planned LSTH from January 2010 until February 2015 were included. Medical records were reviewed for patient characteristics and outcomes.
Results:
One hundred and seven patients were identified. The median age was 46 years (range, 31-59). Preoperative endometrial sampling was obtained in 75 patients (70%). Nineteen patients (18%) had their last cervical smear more than 30 months before surgery. In the only postmenopausal patient of our cohort, the uterus was removed with an extension of the suprapubic skin incision without morcellation. Final histology demonstrated Smooth muscle Tumour of Unknown Malignant Potential (STUMP) in 2 cases and simple endometrial hyperplasia in 1 patient. All 3 patients have had no problems at follow up.
Conclusion:
Even though our cohort was small, there were however 2 unexpected histology results; although none of these patients had any adverse outcomes. Women undergoing LSTH with power morcellation should be appropriately selected and have adequate counselling about the procedure. Our preoperative work up can be improved and should adhere to guidelines in order to maintain safety.

P70- Laparoscopic Supracervical Hysterectomy (LASH): Are we looking for trouble?

Manhal Najdy[1*], Sikhar Sircar[1], Makarand Oak[1], Khalid Elsabagh[1], Mohamed Allam[1]
[1]*Wishaw general hospital, Wishaw, UK*

Objective:
To assess the safety, reproducibility and cost efficacy of LASH.
Methods:
Data was collected retrospectively, 143 women had LASH between August 2008 - November 2014 for benign indications with normal cervical smears and endometrial samples.
Initially one consultant performed the procedure and trained others. 4 consultants are now independently performing LASH.
2 patients towards the end of the study had morecellation in bag to comply with the recent FDA safety advice.
Results:
78% of LASH were performed for AUB, 88.3% had failed treatment. 17% were performed for chronic pelvic pain. The mean operating time was 90 min and mean blood loss was 136 ml.
3.4% had wound infection after LASH however only one pelvic haematoma, one ureteric oedema, one uterovaginal fistula, one hernia from lateral port site and one had scar pain at morcellator porte. No conversion to laparotomy, no blood transfusion, no DVT and no return to theatre.
Average theatre time was 70 minutes for open subtotal hystrectomy and 90 min for LASH. The average running theatre per minute is £3.08, giving £60 more for the LASH.
Instruments for LASH costs £1156 versus £200 for open. The average hospital stay for LASH was 1.7 nights versus 3.7 nights for open. Average cost for one night stay in hospital is £486. Overall cost of LASH was £2259.2 versus £2213.8 for open which obviously comparable with quick recovery and better patient satisfaction for LASH.
Conclusion:
LASH is safe, reproducible, cost effective and quicker recovery and less complication rates.

P71- Laparoscopic surgical approach in premenopausal ovarian cysts

Sudipta Banerjee[1*], Gourab Misra[1]
[1]*University hospital of North Midlands, Staffordshire, UK*

Introduction
As per RCOG guideline, 1 in 10 women have some surgery for ovarian cyst in premenopausal age group.
If surgery is needed, laparoscopic approach is the preferred way
We are reporting an outcome of how benign ovarian cysts were surgically managed in a tertiary care unit.
Methods
30 cases of premenopausal benign ovarian cysts were followed from first clinic appointment till surgery.
Each case was reviewed from history, clinical examination to biochemical and ultrasound markers to detect their suitability for surgical management.
Outcome of surgery and follow up with histology report completed the procedure.
Results
• 27 out of 30 patients had laparoscopic surgery
• 16 of them had laparoscopic cystectomies and 11 had oophorectomy
• All the laparoscopic procedure had an in-patient stay of around 24 hrs
• 3 procedures that had laparotomy started as a laparoscopy
• One had bleeding from pedicle
Two were too large to be removed through ports and both turned out to be dermoid cysts
Conclusion
Laparoscopic approach is the preferred way for surgery of benign ovarian cysts
Preoperative assessment including RMI can determine the success of laparoscopic surgery
Bigger and solid complex cysts, suggestive of dermoid, has a less success of being removed laparoscopically
All women should be informed about a small but important risk of oophorectomy
These factors should be added in when counselling patients preoperatively
Continued training in laparoscopic surgery amongst junior trainees will improve the outcome in laparoscopic benign ovarian cyst surgery.

P72- Laparoscopic treatment of uterine fibroids: *A comparison of peri-operative outcomes of single verses multiple myomectomy*

Ravneet Sirha[1,2*], Reeba Oliver[2,1], Nilesh Agarwal[1,2], Funlayo Odejinmi[2,1]
[1]*Northwick Park Hospital, London, UK;* [2]*Whipps Cross University Hospital, London, UK*

Aims: To compare peri-operative outcomes of laparoscopic myomectomy (LM) for the management of single vs. multiple uterine fibroids.
Methods: Retrospective cohort study of 217 LM performed between 2005 and 2013 at a London teaching hospital.
Results: Length of surgery was significantly greater with multiple myomectomy compared to single myomectomy (128.6 vs 80.5, $p<0.0001$). Multiple myomectomy was associated with increased weight of fibroids (224.1 vs 184.6 $p= 0.0261$), a greater estimated blood loss (333.3 vs 222.8, $p <0.0001$) and an increased use of surgical drains (0.65 vs 0.41, $p = 0.0006$). There was no statistical difference between the rate of surgical complications, drop in haemoglobin, length of hospital stay, resolution of symptoms or patient satisfaction.

Conclusion: Laparoscopic myomectomy for multiple fibroids can be complex and technically difficult surgery. Despite an increase in length of surgery, estimated blood loss and requirement of intraoperative drains, the overall cost effectiveness of a laparoscopic procedure is maintained with no significant increase in hospital stay, requirement of blood transfusions or intraoperative complications. The patient satisfaction and resolution of symptoms is comparable in both groups, suggesting that by an suitably trained surgeon - laparoscopic myomectomy is an appropriate option of management for multiple uterine fibroids.

P73- Learning from Lanarkshire - Informed Consent in Outpatient Hysteroscopy

Katharine Tylko[1*]
[1]*Macmillan CancerVOICE; Cochrane Gynae Cancer Group Consumer Reviewer; James Lind Womb Cancer Alliance member, London, UK*

The Supreme Court in Montgomery v Lanarkshire Health Board (March 2015) ruled that a pregnant diabetic patient should have been informed of a serious risk of shoulder dystocia and been made aware of her right to choose caesarean section, which would have spared her son from cerebral palsy.
The Lanarkshire judgment updates the law regarding informed consent. Doctors are now obliged to inform patients of serious risks and common less serious complications. The judgment quotes the GMC's **'Good Medical Practice**': "Work in partnership with patients. Listen to, and respond to their concerns and preferences."
The presentation will analyse the implications for informed consent regarding the risk of severe pain during specific stages of outpatient diagnostic and/or operative hysteroscopy: cervical dilation, LA injection, electro-surgery, morcellation.
Using data from the **Outpatient versus inpatient uterine polyp treatment for abnormal uterine bleeding: randomised controlled non-inferiority study, 2015**, the presentation will argue that polypectomy patients should be informed of a) the % risk of severe pain and failure during an outpatient procedure and b) the % risk of uterine perforation during inpatient investigation. It is up to each individual patient to assess the risks and benefits and determine her preferred treatment modality. This is consistent with the NHS Choices Hysteroscopy advice.
Numerous patients' responses to the Daily Mail's 2015 article '**NHS doctors who inflict intimate and agonising surgery on women with NO anaesthetic'** will illustrate how the denial of sufficient pain relief could post-Lanarkshire amount to negligence.

P74- Life threatening anaemia secondary to intracardiac metastatic leiomyomatosis

Ilyas Arshad[1*], Rahul Nath[1], Conal Austin[1]
[1]*Guy's and St Thomas' Hospital, London, UK*

Introduction
49 year old Jehovah's witness with a PE on clexane, presented with dyspnoea, per vaginal bleeding and a pelvic mass on examination. She previously had two myomectomies for fibroids. Her haemoglobin was 3 and platelets 25.Our initial impression included that this could be a sarcoma with metastases to the lungs?
Resuscitation was complex without the use of blood and required a multidisciplinary team involving Gynaecology, Haematology and Anaesthetics. Management included: IV immunoglobulins, Methylprednisolone, Romiplostin, Ferrinject, EPO, ferrous sulphate, B12, folic acid and Zoladex. Whilst also being on clexane for her PE.
We diagnosed this mass to be a leiomyomatosis that had metastasised to the heart, it was extending from the IVC to the RA involving the Tricuspid valve.
Definitive surgery was carried out with the gynaecology team carrying out a hysterectomy and the cardiothoracic surgeons removing the intracardiac lesion and repairing the tricuspid valve.
Discussion
This case could be a common scenario that every gynaecology on call doctor may encounter, it illustrates the medical management to treat both severe anaemia and thrombocytopaenia in a Jehovahs' witness, of which there are Leiomyomatosis was first described in 1896 by Birch-Hirschfeld, only 182 cases have been described until 2013. Key factors for suspicion include a lady in her fifth decade of life with a history of having fibroids, even if she has had a hysterectomy or myomectomy as in our case.

P75- Local Anaesthetic for Laparoscopic surgery: Are we doing it right?

Mohamed Shahin[1], Sobha Ramakoti[1*]
[1]*Royal Stoke University Hospital, Stoke on Trent, UK*

Laparoscopic surgical procedures are established to have quicker recovery, less need for analgesia and better patient experience.
As laparoscopic procedures are getting more popular, various techniques and adjuvant methods have been adopted to enhance experience and recovery of the patient from the procedure.
Injection of local anaesthetic is currently widely practiced technique to supplement postoperative analgesia of patients following laparoscopic surgery, however the technique, timing, dosage and choice of agents vary significantly among different laparoscopy teams.
Also, there is rarely a written local guidance or agreement for the best way of providing local anaesthetic. This is usually given to the local preference agreed between the anesthetist and the surgeon.
The aim of the current study is to provide an evidence-based guidance for the best practice of providing local anaesthetic for laparoscopic surgery. A survey is undertaking place to enquire about views, preferences and techniques for local anaesthetic administration. This included: technique, timing, agent, procedure, counseling and appraisal of evidence. This was very useful to gain insight to the variations of practice and willingness for an evidence based guidance.
A comprehensive literature review was done for clinical studies, systematic reviews and basic science reviews, covering different agents and techniques.
There is a need for an evidence-based guidance to be agreed nationally and internationally for the use of local anaesthetic in laparoscopy. We hope that our study will be a helpful starting frame for any further guideline.

P76- Long term self-reported bladder voiding function following surgery for severe endometriosis

Alice Beardmore-Gray[1*], Tom Holland[1], Arvind Vashisht[1], Ertan Saridogan[1], Alfred Cutner[1]
[1]*University College London Hospitals, London, UK*

Aims
Urinary voiding has been noted to be adversely affected in the immediate postoperative period for women undergoing surgery for severe endometriosis. This study aimed to assess the effect of laparoscopic surgery for severe endometriosis on long term self-reported bladder voiding function.
Methods
We retrospectively selected patients with deep endometriosis requiring pararectal dissection who completed both a pre-operative and post-operative (either 6 month, 1 year or 2 year follow-up) BSGE Pelvic Pain Questionnaire. We used patient reported scores from this questionnaire to evaluate urinary voiding following surgery and assess any significant differences by type of surgery.

Results
37/203(18%) patients in our study reported a worsening in score of urinary voiding post-operatively. Of these, 24/37(64%) had a rectovaginal nodule excised, 16/37(43%) underwent bilateral uterosacral ligament excision and 21/37(57%) underwent bilateral ureterolysis. The remaining patients reported either an improvement 28/203(14%) or no overall change 138/203(68%) in voiding function. There was no significant difference noted by type of surgery.
Conclusion
These findings suggest approximately 1/5 women report a worsening in long term bladder voiding function following severe endometriosis surgery. There does not appear to be a correlation between the type of surgery performed and long term self-reported voiding difficulties. However due to the inherent heterogeneity of surgery in these cases, greater numbers of patients may be required to corroborate this. We plan to assess objective urinary voiding tests to further evaluate these important subjective findings.

P77- Measures of success: local experience of an outpatient hysteroscopy service

Helen Nicks[1*], Faizah Mukri[1], George Richter[1], Cinzia Voltolina[1], Shalini Srivastava[1]
[1]*Surrey and Sussex NHS Trust, Surrey, UK*

Nicks, H., Mukri, F., Richter, G., Voltolina, C., Srivastava, S.,
Surrey and Sussex NHS Trust, Canada Avenue, Redhill, RH1 5RH

Introduction
Outpatient hysteroscopy is an effective and safe procedure which prevents the risks of general anaesthesia and minimises the impact of this investigation on a woman's life.
Primary aim
The success rates of outpatient hysteroscopy.
Secondary aims
The percentage of women where a vaginoscopic approach was used. The percentage of women who had an operative procedure successfully. The reason for any failures. The rates of complications.
Method
This was a retrospective six month audit from September 2014 until the end of February 2015 of women attending the outpatient hysteroscopy clinic at Crawley Hospital. The clinic notes and operative diaries used in the outpatient hysteroscopy clinic were analysed. SPSS was used to input and analyse the data.
Results
96% of women successfully underwent the procedure. 98% of women underwent the procedure vaginoscopically. The commonest reason for failing were cervical stenosis and pain. 2% of women went on to require a hysteroscopic procedure under general anaesthetic. There were no uterine perforations and the rate of complications was low.
Conclusion
The results of this audit are very promising that we are delivering a successful service. However further work on the development of operative procedures such as ablation is needed to reduce the number of women needing day case surgery under general anaesthetic.

P78- Measuring outcome of Laparoscopic hysterectomy in a university hospital

Sudipta Banerjee[1*], Gourab Misra[1]
[1]*University hospital of North Midlands, Staffordshire, UK*

Introduction
NICE guidance 2007 suggests that safety and efficacy of laparoscopic techniques for hysterectomy appears adequate to support their use, provided that arrangements are in place for consent, audit and clinical governance.
As a teaching university hospital we are moving towards laparoscopic hysterectomy than conventional open surgery.
We are reporting the outcome of laparoscopic hysterectomy in our department.
Methods
34 cases of laparoscopic hysterectomy were reviewed.
Cases were studied from consenting for the procedure to a follow up of 6 weeks postoperative period.
The main outcomes were
• Indication
• Preoperative counselling
• Level of surgeon
• Length of inpatient stay
• Rate of conversion and reason
• Complications (blood loss, visceral injury)
• Return to normal activity at 6 weeks follow up
Result
There were 23 total laparoscopic, 8 laparoscopic assisted vaginal, 1 subtotal laparoscopic and 2 open hysterectomies (converted from TLH)
Main indications were endometriosis and endometrial hyperplasia, fibroids and pelvic pain
Three different consultant teams were reviewed
Two third of procedures were performed by consultants and one third by senior trainees.
Our departmental conversion rate was 2/34, which is 5.9%
There has been no bowel, visceral or major vascular injuries
Rate of bladder and ureteric injury were high (around 3%)
Conclusion
• Proper preoperative counselling regarding urinary tract injuries, rate of conversion, haemorrhage and visceral and vascular injuries are essential
• Early and delayed complications need diagnosis and management aggressively to prevent adverse outcome
• Adequate training is key to the success rate and prevention of complications

P79- Missed opportunities for total laparoscopic hysterectomy

Jane Borley[1,2*], Robert Richardson[1]
[1]*Chelsea & Westminster NHS Foundation Trust, London, UK;* [2]*Imperial College, London, UK*

Introduction
Total laparoscopic hysterectomy (TLH) is associated with less pain, shorter hospital stay and quicker recovery. It is widely advocated to be the preferred method over total abdominal hysterectomy (TAH) when possible, but despite this there are still some women who are not offered the laparoscopic approach.
Aim
To determine the number of women who underwent TAH where TLH may have been possible.
Methods
A retrospective audit on women undergoing TAH from 1st January 2014 to 31st December 2014 for benign disease. Women with suspected/confirmed malignancy or large ovarian masses were excluded.
Results
60 patients were identified who underwent TAH in 2014. Indications for hysterectomy were menorrhagia or pressure symptoms caused by uterine fibroids - 41 patients (68.3%), dysfunctional uterine bleeding - 15 (25%), adenomyosis/pain - 2 (3.3%), recurrent CIN - 1 (1.7%), postmenopausal bleeding - 1 (1.7%).
In 41 patients undergoing TAH for fibroids, 33 cases (80.5%) had a uterine volume of ≥12/40, with a median of 17/40. Of those <12/40 size (n=8), only 2 had known contraindications for TLH (extensive pelvic

adhesions with planned colorectal surgical involvement and uterus too large at laparoscopy).
19 patients had TAH without fibroids, all of these were <12/40 size. 14/19 cases (73.7%) did not have an obvious contraindication for laparoscopy. Contraindications were extensive pelvic and bowel adhesions in 3 cases and patient request for abdominal approach in 2 cases.
Conclusion
20 patients (33.3%) who underwent TAH for benign disease may have been suitable for a laparoscopic approach, this approach should always discussed and considered preoperatively.

P80-*Modified Palmer's Point (MPP)* : *A New Alternative Laparoscopic Initial Entry Point*

Raphael Laiyemo[1*], Stewart Disu[2]
[1]*Kings Mill Hospital, Nottinghamshire, UK;* [2]*NorthWick Park Hospital, London, UK*

Introduction
Intra-umbilical entry is the traditional laparoscopic initial entry point to establish pneumo-peritoneum used by most gynaecologists.
Laparoscopic surgeon should have an alternative entry point in patients with previous abdominal surgery involving paramedian, midline or paraumbilical incisions. The risk of bowel and omental adhesions to the umbilical region is significantly higher in this group of patients.
Furthermore, after 2 or more failed intra-umbilical entry attempts, very high or low BMI patients, an alternative entry point like Palmer's point or Lee-Huang point should be considered. This minimises complications such as pre-peritoneal insufflation and injury to intra-peritoneal structures.
Palmer's point, an entry point at the mid-clavicular line 3cm below the left subcostal margin, was first described by Raoul Palmer. However, Palmer's point as originally described falls within the anatomical zone of the superior epigastric artery as shown by Saber et al in 2004.
Technique
Start by measuring 8cms laterally from the midline just above the level of the umbilicus (Point A). A second line is drawn vertically from 'Point A' to 3cm short of the left subcostal margin (Point B). 'Point B' is the Modified Palmer's Point (MPP).Entry at this point avoids any potential injury to the superior epigastric artery. MPP entry has an equal puncture:pneumo-peritoneum (P:P) ratio as Palmer's Point.
If significant caudal displacement of umbilicus due to morbid obesity is present, Pelosi re-alignment technique should be done before using MPP.
Conclusion
MPP is a new safe alternative entry point which avoids the superior epigastric artery. It is reliable, accurate and easily reproducible.

P81- Morbidity at Laparoscopic Surgery

Deepa Balachandran Nair[1*], Ganashelvi Premkumar[1], Franz Majoko[1], Adnan Bunkheila[1]
[1]*Singleton Hospital, Swansea, Wales, UK*

Introduction
The reported incidence of complications of laparoscopic surgery varies between 1-12.5/1000 and is dependent on complexity of the procedure and experience of the operator. We conducted this audit to examine entry techniques, indications & complication rates of laparoscopic surgery in our department.
Methods
This was a retrospective audit conducted at Singleton Hospital, Swansea. Ninety women who had laparoscopic surgery were selected from the hospital database. Case notes were reviewed when additional information was required. Standards for comparison were derived from the BSGE guideline and a recent Cochrane review.
Results
Twenty one percent of women (19/90) had a previous surgery. Veress entry was used in 90% of cases (81/90). In women with previous surgery, the Veress entry was still preferred in 74% of cases (14/19). Pelvic pain was the most common indication. 79% (71/90) were elective procedures. There were 3 cases of haemorrhage from the secondary port site. There were no major complications.
Discussion
It was recognised that the hospital database lacked a means of capturing intra-operative complications. Recommendations were made to capture this information electronically. A new theatre database system was introduced, that records risk factors and complications allowing prospective monitoring of laparoscopic surgical morbidity.
There was a distinct tendency to use the Veress entry. Recommendations were made to make use of other methods of entry. Training to enable surgeons to gain experience in different forms of entry was started.

P82- Mutidisciplinary Meeting for Endometriosis- An approach to optimise Patient outcomes

Suku George[1*], Joanne Dzyra[1], Sajal Rai[1], Andrew Pickersgill[1], Maryna LLewinsky[1]
[1]*Stockport Endometriosis Center, Stockport, UK*

Methods
Stockport Endometriosis Centre was accredited by the BSGE in 2013. From its inception a multidisciplinary meeting was set up to have a formal consensus over management and to make individualised patient plans. This was modelled on the Gynaecological Oncology MDT meeting and takes place in a video conferencing room with ability to link various hospitals in the region. Monthly meetings are held with Radiologist, Gynaecological surgeons, Colorectal surgeon and Endometriosis nurse with outcome and discussion documented on a dedicated proforma and communicated to GP and patient. Level of Colorectal/ Urology involvement, Postoperative follow up and imaging are agreed at MDT.
Clearly defined referral criteria such as
Clinical or radiological suspicion of Rectovaginal disease
MRI showing visceral involvement
Lateral disease with distal ureteric involvement
Post hysterectomy disease
Recurrent disease after complex surgery
Post operative discussion to close MDT loop
MDT makes consensus recommendations for treatment modality, further investigations and level of multidisciplinary involvement. Colorectal support is decided as either assistance or joint procedure.
When joint procedure is planned with high probability of bowel resection, preoperative meeting with stoma nurses is arranged and patients are admitted day before surgery in the surgical ward with bowel prep and enhanced recovery pathway.
Results
135 cases discussed over past two years and 54 were categorized as severe endometriosis. With 5 patients currently awaiting surgery 46 patients (94%) were correctly identified with pre operative MRI imaging . 2 cases (4%) were under called on MRI and one patient (2%) had disease volume over diagnosed.

P83- New outpatient hysteroscopy services set up: lessons learned

Eve Gaughan[1*], Fani Gkrozou[1], Bronwyn Middleton[2], Richard Pyper[1], Natasha Waters[1]
[1]*Southlands Hospital, West Susssex, UK;* [2]*St Richard's Hospital, West Sussex, UK*

Introduction: Our out-patient hysteroscopy clinic is a new service for our trust and we reflect on lessons learnt after the first year of its existence.
Material and Methods: All staff were interviewed regarding lessons learnt over the course of the year with a view to providing a safe and efficient service associated with the highest possible patient satisfaction scores. We performed a 6 month audit of our service and analysed data from a questionnaire given to 74 women after outpatient hysteroscopy.
Results: Clinic protocols for patient assessment, procedure performance and management of potential complications are necessary to ensure an efficient and safe service.
The collection of patient feedback is invaluable in helping to inform and improve the service
Patient leaflets given before attending were beneficial in terms of improving pain and patient satisfaction scores. Reminding patients in their appointment letter of the importance of to reading the leaflet and taking analgesia was found to be helpful.
Patients waiting over 30 minutes pre-procedure increased anxiety levels and increased pain scores. Appropriate timing and duration of appointments improved patient satisfaction rates-
Uniform pregnancy testing in all premenopausal women and deferring procedures in the luteal phase in those without appropriate contraception should be the policy
Strategy for difficult entry cases with extra equipment readily available
Vaginoscopic technique is default approach.
Importance of the same team of nurses familiar with equipment and procedures who can trouble shoot and ensure efficient set up to which help to reduce patient anxiety

P84- Obesity and subfertility: identifying the challenges faced by the subfertility specialist and reproductive surgeon

Sophie Elizabeth Mayhew[1*], Shamma Al-Inizi[1]
[1]*South Tyneside District Hospital, South Tyneside, UK*

Background: Obesity is a well documented risk factor for subfertility; impacting ovulation, eqq quality, implantation and miscarriage rate. Additionally, treatment of this subgroup of patients may pose further challenges in the form of technically difficult procedures, treatment resistance and poor patient engagement.
Methods: we retrospectively evaluated the management of 100 women with a body mass index (BMI) >30kg/m2 attending subfertility clinic, analysing procedure failure and laparoscopic complication rate, ovulation induction and drilling rate, successful weight-loss and pregnancies achieved.
Results: BMI ranged from 30-60kg/m2. Weight loss was achieved in 35 (35%) of patients. 39 (39%) became pregnant and 17 (43%) of those had lost weight. 10 (10%) fell pregnant following advice alone. 67 (67%) of women were anovulatory and 17 (25.4%) of those were resistant to Clomiphene Citrate. 12 (16.4%) required ovarian drilling. 8 (11.9%) of anovulatory women were too overweight to safely commence Clomiphene Citrate. For 6 (6%) women, the cervix could not be visualised and/or cannulised at hysterosalpingogram leading to laparoscopy. 3 (10%) of laparoscopies converted to open laparoscopy using Hassan's method due to difficult entry. 35 (35%) of women were lost to follow up without achieving pregnancy.
Conclusions: Obesity can present many challenges to subfertility treatment including treatment resistance and procedure failure. However, appropriate counselling and support alone may often lead to successful weight loss and pregnancy.

P85- Observational Study of Outpatient Hysteroscopy Outcomes Amongst Postmenopausal Women in a UK District General Hospital

David Ankers[1*], Lynda Coughlin[1], Ajay Swaminathan[1]
[1]*Mid Cheshire Hospitals NHS Foundation Trust, Crewe, UK*

Ankers D, Coughlin L, Swaminathan A
Mid Cheshire Hospitals NHS Foundation Trust
Introduction
Outpatient hysteroscopy is a well established technique for the investigation of postmenopausal bleeding. The appropriate use of miniature sized hysteroscopes can allow good visualisation of the endometrial cavity, facilitate image guided biopsy, polypectomy and also avoid the risks of general anaesthesia (1,2).
Methods
Retrospective case note review over an 18 month period. Cases identified via hospital information services department.
Results
89/90 patients who had undergone hysteroscopy had postmenopausal bleeding. 81/90 patients had an endometrial thickness above 3mm not on hormone replacement therapy. There was a 97.5% success rate of outpatient hysteroscopy during the sample period. The rate of malignancy identified on histology was 14/90 (15.5%), with 5/14 (35%) highlighting endometrial adenocarcinoma. There was also one case of carcinosarcoma and papillary serous carcinoma during this timeframe.
Conclusions
Our results indicate that most patients had an appropriate endometrial thickness for hysteroscopic assessment. We also found that our rate of malignancies identified from endometrial sampling is comparable with other research. Further investigation is required to study prevalence of rarer malignancies such as carcinosarcoma.
References

- 1. Royal College of Obstetricians and Gynaecologists.Green Top Guideline No.59: Best Practice in Outpatient Hysteroscopy. London:RCOG; 2011
- 2. Marsh F,Kremer C,Duffy S.Delivering an effective outpatient service in gynaecology.A randomised controlled trial analysing the cost of outpatient versus daycase hysteroscopy.BJOG 2004;111:243-8.

P86- 'One Stop' Hysteroscopy Clinic: A Patient Survey

Caroline Smith[1*]
[1]*NHS Greater Glasgow and Clyde, West of Scotland, UK*

Objectives:
Identify the percentage of women receiving written information prior to appointment.
Evaluate patient experience and pain scores for scan, hysteroscopy and endometrial biopsy.
Data collection:
Survey given to patients following their visit to the one-stop clinic at Clinic F, Stobhill ACH from June to September 2014. 77 surveys were returned.
Results:
The majority of patients received an information leaflet in advance, found it easy to understand, and felt it made their visit easier.
Patients who underwent hysteroscopy had a mean pain score of 3.1, whereas those who had a biopsy scored 4.3.
Almost all those surveyed (97%) would recommend the service to a friend.
Discussion:
The clinical and economic benefits of a ‘One Stop' clinic for the management of abnormal uterine bleeding are well recognised and in general the procedures involved are well tolerated1. However it is important that all efforts are made to reduce patient anxiety as this can decrease the likelihood of successfully completing all relevant procedures2. Patient information leaflets should therefore be provided to help prepare patients.

The most striking finding of this survey was the overwhelmingly positive impact of the clinic staff on the patient experience.
In conclusion, the vast majority of patients attending the One Stop Clinic at Stobhill have a positive experience and tolerate all necessary procedures well. The support of the dedicated staff is invaluable and significantly contributes to the overall perception of the service.
1. RCOG Green Top Guideline No.59
2. Surg Endosc. 2004 Jul;18(7):1099-104. Epub 2004 May 12

P87- One-stop out-patient hysteroscopy service reduces time to definitive investigation in women referred with Post-menopausal bleeding

Christopher Brewer[1*], Carole Wilson[1], Claire Oxby[1]
[1]*York Teaching Hospital NHS Foundation Trust, York, Yorkshire, UK*

Introduction.
Post-menopausal bleeding (PMB) may signify the presence of endometrial cancer, 10% with PMB under 60 years will have endometrial cancer; rising to 13% over 60 years. The Cancer Reform Strategy (2007) recommends that patients with a suspected cancer should be referred urgently to secondary care and 1st hospital assessment should take place within two weeks.
We sought to determine whether the introduction of a one-stop out-patient hysteroscopy clinic for women with PMB and increased endometrial thickness (ET>4mm) reduced referral to attendance interval.
Methods.
A retrospective audit comparing of a two month period in 2011 following the historical "PMB clinic" model (November 2011-December 2011) and a two month period in 2012 following the "Out-patient hysteroscopy clinic" model (August 2012-September 2012).
Data regarding referral times, scan and clinic visits, and outcomes were drawn from the hospital clinical database.
Results.
In the study period, 89 women attended the "PMB clinic" and 111 in the "Out-patient hysteroscopy clinic" model.
The "PMB Clinic" model had an average referral to assessment interval of 11 days (95% CI 9.75-12.25d). However, patients with increased ET then went on to receive urgent hysteroscopy, as such mean time from referral to definitive investigation was 48.2 days. The "OP hysteroscopy clinic" model had an average referral to assessment interval of 15.4 days (95% CI 11-19.78d), 13.41 days (95% CI 11.58-15.24d) with the exclusion of a 127d outlier.
Conclusion.
The one-stop out-patient hysteroscopy model was associated with a reduced interval from referral to definitive investigation in women PMB.

P88- Opportunistic Bilateral Salpingectomy (OBS) for prevention of ovarian cancer: support for a clinical trial or routine care amongst UK clinicians

Ranjit Manchanda[1,2], Dhivya Chandrasekaran[3*], Ertan Saridogan[4], Robin Crawford[5], Eleanor Brockbank[1], Jatinder Kalsi[2], Davor Jurkovic[4], Usha Menon[2]
[1]*Department of Gynaecological Oncology, St Bartholomew's Hospital, London, UK;* [2]*Department of Women's Cancer, EGA Institute for Women's Health, University College London, London, UK;* [3]*Department of Obstetrics and Gynaecology, Northampton General Hospital, Northampton, UK;* [4]*Department of Gynaecology, University College London Hospital, London, UK;* [5]*Department of Gynaecological Oncology, Addenbrookes Hospital, Cambridge, UK*

Background
Opportunistic Bilateral Salpingectomy (OBS) is being advocated as an ovarian cancer (OC) prevention strategy at the time of benign gynaecological surgery/tubal ligation for premenopausal women who have completed their family. We report on the prevalence of OBS in current practice and its acceptance amongst UK clinicians.
Methods
An anonymised web-based survey was sent via the RCOG monthly e-newsletter. Baseline characteristics were described using descriptive statistics. Chi-square test was used to compare categorical, Kendal-tau-b test for ordinal and Mann-Whitney test for continuous variables between two groups.
Results
Of the 395/4000(9.9%) respondents, 81% were general obstetricians & gynaecologists and 17% subspecialists. 61% agreed with the tubal hypothesis, 33% performed OBS 'always/most of the time' and 50% supported its introduction. However, 53% thought OBS should 'only' be offered within a clinical trial with 89% willing to support such a study. Lack of data on OC risk reduction(78%), RCT evidence of benefit(76%), and impact on ovarian function(65%) were the leading factors limiting its introduction into routine clinical practice. Maximum support for OBS was at the time of hysterectomy (92%) and tubal ligation (65%). Training implications for self/trainees were highlighted by 21%/49% respectively.
Conclusion
There is broad support in the UK for the principle of OBS with equivalent support for introduction 'only' within a trial/ within clinical practice. The need for prospective evidence to validate its efficacy was highlighted with 89% willing to support a clinical trial. A randomised trial powered on menopause outcomes may be a way forward.

P89- Outpatient hysteroscopy versus ultrasound in abnormal uterine bleeding - should we scope everyone?

Emma Abery[1*], Lois Delchar[1]
[1]*Barking Havering and Redbridge NHS Trust, London, UK*

Background : Hysteroscopy is considered the gold standard in diagnosing intrauterine pathology in women presenting with abnormal uterine bleeding (AUB) and post menopausal bleeding (PMB) suggestive of endometrial pathology. Routine practice in our unit is to carry out ultrasound (US) in all women with AUB, only proceeding to hysteroscopy for recurrent episodes, if US suggests pathology or there is high clinical suspicion.
Method : We carried out a prospective audit to compare US, outpatient hysteroscopy findings and final histopathology results in women referred with abnormal uterine bleeding.
Results : 98 patients, aged 36-91 were seen for outpatient hysteroscopy. 75 had PMB, 5 were perimenopausal, 3 had incidental endometrial thickening and 15 had other AUB. 5 had serious pathology detected (3 endometrial hyperplasia; 2 endometrial cancer).
Hysteroscopy revealed intrauterine pathology in 62% of cases, with 1 false negative where the cavity appeared atrophic in the presence of simple hyperplasia on biopsy. US was normal in only 5 women, of whom 2 had benign pathology found at hysteroscopy. The sensitivity of US to detect pathology was 96% but it had only 11% specificity. We found the ultrasound reports had heterogenous descriptions, making it difficult to identify whether serious pathology may or may not be present.
Conclusion : If US is to be considered a useful tool for investigating AUB, stricter guidance should be used for reporting findings, otherwise with a specificity of only 11% it has been proposed that we save the cost of an ultrasound and offer a hysteroscopy to everyone.

P90- Patient feedback on outpatient hysteroscopy in a busy district general hospital – The importance of improving patient care in ambulatory gynaecology

Fiona Cowan[1*], Vidya Shirol[1], Annabelle Burnham[1]
[1]*Frimley Health NHS Trust, Surrey, UK*

The RCOG guideline 'Best Practise in Outpatient Hysteroscopy' states that 'all gynaecology units should provide a dedicated outpatient hysteroscopy service to aid management of women with abnormal uterine bleeding'. Recommendations include the use of appropriate facilities outside a formal operating theatre setting, written patient information and consent taking prior to the procedure and ways to decrease pain scores including the use of miniature hysteroscopes, vaginoscopy and encouraging NSAIDs one hour prior to the procedure.
Objectives: To assess patient satisfaction and whether further improvements were required to the service provided in our unit.
Methods: 100 patient satisfaction questionnaires were completed between June 2014 and November 2014.
Results: 100% of patients believed the clinic was easily accessible, staff professional and privacy and dignity maintained. All patients surveyed believed they were given suitable information prior to the procedure with adequate aftercare. 87% received information leaflets prior to the procedure. 84% of patients were waiting for their procedure for less than 30 minutes with 93% finding self -check in kiosks beneficial. 76% had only mild to moderate discomfort. 93% would have outpatient hysteroscopy again, 89% recommending the procedure.
Conclusion: with excellent patient satisfaction, outpatient hysteroscopy should be encouraged in suitable candidates. Further improvements to clinic set up and facilities should be ensured and audited across all units. Information evenings could be arranged for General Practitioners and better dissemination of patient information leaflets prior to outpatient procedures should be encouraged to further improve care and satisfaction.

F91- Patients' perspective of the outpatient hysteroscopy service: a patient satisfaction survey

Faizah Mukri[1*], Helen Nicks[1], George Richter[1], Shalini Srivastava[1], Cinzia Voltolina[1]
[1]*Surrey and Sussex NHS Trust, Surrey, UK*

Introduction
With a move towards outpatient hysteroscopy as a first line for women it is important to ensure the best experience during this procedure.
Primary aim
The percentage of women who were satisfied with the service and would recommend it to a friend.
Secondary aims
The percentage of women who received information pre procedure. The percentage of women who were satisfied with the reception, staff, and after care. The experience of women during their visit to clinic.
Method
This was a prospective patient survey, given to all patients attending the clinic between December 2013 and February 2014. Qualitative and quantitative data was analysed.
Results
The response rate was 53%. 100% were satisfied with the experience and would recommend to a friend. 100% felt they were treated with dignity and respect. However, only 78% were given pre-procedure information. 8% were not satisfied with the reception area and staff.
Conclusion
The results of this audit are very promising that we are delivering a successful service which is well received by our patients. However it has highlighted areas for continued improvement including pre-procedure information and the welcoming area.

P92- Post Menopausal Endometriosis, A Rare Case Report

Chandrakala Sathish[1*], Matteo De Martino[1], Stanley Okolo[1]
[1]*North Middlesex University Hospital, London, UK*

Introduction: Endometriosis is one of the most common gynaecological disorders that affects women in reproductive age and postmenopausal age. It is defined as the abnormal implantation of the glandular epithelium or the endometrial stroma at extrauterine sites. Endometriosis can be misdiagnosed and thus pose a diagnostic predicament. Diagnosis is generally made after Laparoscopy and histological examination.
Case presentation: We present a case of postmenopausal endometriosis in a 41-year-old woman with history of recurrent endometriosis despite undergoing hysterectomy and bilateral salpingoophrectomy (BSO). Postmenopausal endometriosis is uncommon, since after the cessation of menstruation, production of ovarian oestrogen ceases. Although a number of such cases have sporadically been reported the present state of the data is insufficient to permit any appraisal of this and the mechanisms fundamental to the entity have not been thoroughly explained.
Here we discuss about pathophysiology associated with recurrent endometriosis in postmenopausal status, especially the activity of Aromatase P450 enzyme, which is present in the endometriotic tissue itself producing estrogen and hence a positive feedback causing endometriosis in postmenopausal women without any need for external estrogen.
Laparoscopic excision has been recommended for such cases because it reduces the risk of possible malignant transformation in such cases. Administration of aromatase inhibitors has also shown success in suppressing local and exogenous oestrogen, but there is still a need of further research to validate its clinical uses.
Conclusion: Although the reported situation is uncommon, it is vital to be aware of post-menopausal endometriosis because it confers a threat of malignant transformation (0.7-1%).

P93-Prognostic factors that predict success in office endometrial ablation: a cohort study

Paul Smith[1*], Shilpaja Karpate[1], Justin Clark[1]
[1]*Birmingham Women's Hospital, Birmingham, UK*

The objective of the cohort study was to identify clinical factors that influence the rate of further surgical intervention in women who had endometrial ablation. Prospectively held electronic databases and patient records were scrutinised to obtain historical, examination, investigative and procedural data considered to be potentially predictive of the need for further surgical intervention after endometrial ablation in the office setting. A total of 391 consecutive women were identified who received endometrial ablation in the office setting between July 2005 and December 2012, with an average follow-up of 4.3 years. Univariable and multivariable logistic regression were used to estimate the influence of these variables on prognosis. Factors predictive of further surgical treatment were dysmenorrhea (aOR 4.01; 95% CI 1.63 to 9.91) and a uterine cavity length >9cm OR (aOR 2.65; 95% CI 1.33 to 5.27). In conclusion, dysmenorrhoea before treatment or a uterine cavity length >9cm are associated with the need for further surgical interventions after office endometrial ablation. These findings should help inform clinician and patient decision making when considering treatment options for heavy menstrual bleeding.

P94- Rate of laparoscopic skill acquisition in novice students

Dominic Harrison[1], Jessica Stephen[1*], Jamie Burrell[2], Emma Chang[2], Edward Christopher[2], Ellie Couser[2], Shivali Dawda[2], Sasha Gold[2], Iain Hettle[2], Rory Thompson[2], Kathryn Young[2], Tony Yu[2], PaulMills[1]
[1]*St John's Hospital, Livingston, UK;* [2]*University of Edinburgh, Edinburgh, UK*

This study aims to investigate the rate of laparoscopic skill acquisition on a simulator to gain insight into the inherency of these skills and potential for their improvement.

Ten first year medical students with laparoscopic experience were timed at five-minute intervals over an hour as they completed a task on a simple laparoscopic simulator. Their initial times were compared with those of obstetrics and gynaecology registrars (ST3+) with no experience of the task, but laparoscopic experience.
The mean recorded initial time of trained registrars (n=9) (191 seconds (range 90-332s)) was significantly shorter than novice students (n=10) (452 seconds, (range 208-787s). The mean time taken for novices to complete the simulated task decreased with time; mean completion time had decreased by 42.18% after 5 minutes of practice, 62.0% after 10 minutes, 74.2% after 25 minutes, and 80.6% after 65 minutes.
The results suggest an overlap between the skills developed through laparoscopic surgery and those required to complete the simulated task as registrars and students were differentiated by skill level. The mean time that novices took to complete the simulated task decreased over time, as a result of increased familiarity with the task's skill requirements such as depth perception and dexterity, and tended towards a plateau. Further work will be needed to see if increased access to these inexpensive simulators for registrars may improve their rate of skill acquisition and if this is transferable to their performance in theatre.

P95- Relation between body mass index and patient outcomes in total laparoscopic hysterectomy: a retrospective observational study in benign and cancer cases

Vasileios Minas[1*], Claudia Ventii[1], Nicola Murray[2], Thomas Aust[1], Nahid Gul[1], David Rowlands[1]
[1]*Department of Obstetrics & Gynaecology, Wirral University Teaching Hospital, Upton, Wirral, UK;* [2]*School of Medicine, University of Liverpool, Liverpool, UK*

Increasing numbers of gynaecologists are offering the benefits of laparoscopy to obese patients. Obesity is a risk factor for peri-operative morbidity across surgical specialties. Limited research has been conducted on the effect of obesity on outcomes of major laparoscopic pelvic surgery. Some authors report worsening outcomes in obese women having laparoscopic hysterectomies. Others suggest that complication rates do not increase, but operating times are longer.
Here we report our experience from 252 total laparoscopic hysterectomies performed for both benign conditions and gynaecological cancers, and we compare outcomes among normal, overweight, obese and morbidly obese patients. We used a composite score index calculated on the basis of operating and theatre times, estimated blood loss, length of stay, and number and severity of complications.
The mean BMI of our patients was 30.8 and mean age was 52.9 years. The highest mean composite score was observed in morbidly obese patients. In these patients operating and theatre times were longer. Overweight and obese patients' mean scores were lower than normal weight patients, but statistical analysis failed to show significant differences with the exception of morbid obesity. Regression analysis showed no relationship between the two variables.
Our study has a number of limitations including the inherent bias of a retrospective observational study and a relatively low number of cases. Still, it suggests that meticulous, consistent surgical technique may produce similar outcomes in normal and obese patients having total laparoscopic hysterectomy, with longer operating/theatre times noted only at BMI levels >40.

P96- Review of 6 months data of outpatient hysteroscopy episodes at West Cumberland Hospital; setting up the standards of Care, philosophy of the service and defining outcome measures

Oudai Ali[1*], Haissam Moukarram[1]
[1]*West Cumberland Hospital, Whitehaven, UK*

Introduction
Outpatient hysteroscopy is very valuable, particularly in managing postmenopausal bleeding. A failed episode will happen in case of the following;

- inability to proceed with the procedure after premedication
- failure to access the cavity after proceeding
- inability to remove pathology after accessing the cavity
- causing severe pain with unsatisfactory experience

Methods
74 actualised hysteroscopies out of 85 referrals for a newly set outpatient hysteroscopy service were analysed. The practice was supported by a thorough recording of many parameters and outcome measures through prospective audit together with patient satisfaction survey including pain scores. The local anaesthesiology pathway included, if tolerated, patients taking paracetamol and ibuprofen with a light meal before coming to their appointments. The majority on arrival will be given, tramadol, buscupan, ranitidine and cyclizin.The cervix is infiltrated with 4-6 injections of lignospan and instillagel is used for lubrication. If there is pathology then the cervix is dilated to deal with it.
Results
The pick up rate of significant pathology was about 47/74 and the pickup rate of focal pathology was 31/74.There were 7 cancers diagnosed and 6 pre cancers of the endometrium. The cavity was accessed in 100% of cases but there were three failures to remove pathology. Complications were limited; one cervical trauma and 4 cases vagal reaction. Only two cases reported unsatisfactory experience. The procedure's average duration was less than 15min in 85% of the cases
Conclusion
Outpatient hysteroscopy service is feasible, safe with high satisfaction rate and proved valuable in picking up significant pathology. It is essential to define the philosophy of service and reflect its performance through prospective audit.

P97- Review of outcome following hysteroscopic division of intrauterine adhesions or uterine septum

Dona Edirisinghe[1*], Ephia Yasmin[1]
[1]*University College Hospital, London, UK*

Background: Hysteroscopic procedures are well tolerated by the patients and effective in treating intrauterine pathology. Systemic reviews did not find any significant difference in reproductive outcome following resection of intrauterine septum and adhesions. However, small observational studies have found beneficial effects.
Method: An observational study of women, who has undergone either resection of intrauterine septum or intrauterine adhesions during the period of 3 years from 2011 till 2014 at University College Hospital, London.
Results: Eleven women were identified for the study. Seven women had intrauterine adhesions following either previous delivery or surgical evacuation of miscarriage. Four women had uterine septum, while two of them had recurrent miscarriages. Following resection of septum and division of adhesion three women needed repeat procedure. Two women had successful pregnancies with IVF treatment and spontaneous conception. Three women had early pregnancy losses and one had failed IVF. Three women who needed repeat procedure are awaited for formation of normal endometrium prior to assisted conception.
Conclusion: There is no significant improvement in fertility outcome following the procedure. This may related to other contributing factor such as age and premature ovarian failure.

P98- Safe entry Techniques; Patient selection and Risk Factors Assessment in Tailoring Individual Patient-Based Laparoscopic approaches

Karim Abdallah[1*]
[1]Royal United Hospital NHS Foundation Trust, Bath, UK

Minimal access surgery is one of the hall marks of this day and age of gynaecological surgery. Laparoscopy is progressively taking over most of the procedures. Different access techniques have been discussed in more than several occasions. Assessing the individual risk factors of each patient is an absolute necessity in the decision-making process on the best way of gaining safe entry into the abdominal cavity. We aim at reviewing different entry techniques described in the literature and highlighting different individual risk factors that should be taken into consideration when choosing the entry technique. We aim at providing a simple guidance to help safe decision -making on the choice of entry technique to be used. The evidence will be drawn from English-language articles published over the last decade on Medline, Pubmed and the Cochrane data base.

P99- "See & Treat" Satisfaction - Procedural Outpatient Hysteroscopy @ St Richard's Hospital Chichester

Gina Michel[1*], Bronwyn Middleton[1]
[1]St Richard's Hospital, Chichester, UK

All patients attending the See & Treat outpatient hysteroscopy clinic are asked to complete an anonymous patient satisfaction survey following their appointment to support service improvement and development. All patients are given an information leaflet prior to their appointment about what to expect, pre-procedural analgesia, after care and alternative treatment options including general anaesthetic. Each clinic has a dedicated team with a list of 6-8 patients, each with a half hour slot. Diagnostic and procedural hysteroscopies are performed.
Data from surveys collected from 150 women over 6 months regarding demographics, patient information leaflets, appointment waiting times, pain scores, and experience and satisfaction rates were put onto a spreadsheet and analysed.
93% of women received a patient information leaflet prior to their appointment, of which 98% found this to be 'very informative' or 'informative'. 84% of women reported that the information leaflet relieved their anxieties partially or completely prior to the procedure.
A third of women did not experience any adverse symptoms after the procedure. The most common symptom after the procedure was abdominal cramps, which 47% of women reported. A minority of others experienced shoulder tip pain, nausea and feeling faint.
Overall there was a 99% satisfaction rate with 51% of women reporting their experience was better than expected and 40% as expected. 91% rated their care as excellent.
Analysing patient feedback is imperative to service development and improvement and forms an integral part of our Trust's Patient First initiative.

P100- Size of submucous fibroid and success of TCRF

Rubab Ali[1*], Dimitrios Mavrelos[1], Ertan Saridogan[1]
[1]Reproductive Medicine Unit, University College London Hospital, London, UK

Introduction:
Submucous fibroids are associated with menorrhagia and adverse reproductive outcome. Transcervical resection (TCRF) is an effective operation to remove these tumours. We have shown that women with a submucous fibroid >38mm diameter are more likely to require a repeat TCRF. This study aimed to audit our practice and validate this cut off.

Methods:
A retrospective 5-year observational study. Primary outcome measure was complete fibroid resection. Secondary outcome measures were operative fluid deficit, symptom resolution and complication rate.
Results:
During the study period 91 patients underwent TCRF. On ultrasound 74/91 (81%) women had a single fibroid and the median diameter of the largest fibroid was 25mm (15mm IQR). On hysteroscopy 38/78 (49%) fibroids were Type 2, 15/78 (19%) fibroids Type 1 and 25/78 (32%) fibroids Type 0. 50/78 (64%) fibroids were completely resected at a single operation. Median fluid deficit was 275 ml (range 0 – 3000), 3/78 (4%) women received diuretics for suspected fluid overload and 1/78 (1%) required an intrauterine Foley catheter. 63/80 (79%) women had symptom resolution.
37/48 (77%) fibroids <38mm diameter were completely resected at a single operation vs. 3/8 (38%) fibroids ≥38mm (p=0.022). Those that had incomplete resection 7/16 (44%) had a repeat TCRF. 9/16 (56%) had sufficient improvement in symptoms.
Conclusion:
TCRF is an effective and safe procedure that improves symptom. A cut off of 38mm for the preoperative diameter of the largest fibroid can predict need for a repeat TCRF.

P101- Spinal abscess as a complication of ultrasound guided per-vaginal drainage of hydrosalpinx in a patient with a background of Marfan Syndrome

Tim Hookway[1*], Sophie Kay[1]
[1]St George's Hospital, Tooting, London, UK

Introduction
We present an unusual case of spinal abscess as a complication of ultrasound guided per-vaginal drainage of hydrosalpinx.
Case Report
A 56 year old woman with a background of Marfan's syndrome and metallic aortic heart valve underwent an elective repeat transvaginal ultrasound guided drainage of left hydrosalpinx under local anaesthetic.
Following the procedure she became pyrexial with worsening pelvic and back pain and cycling tachycardia. Intravenous antibiotics were initiated for probable pelvic infection. Inflammatory markers were raised with negative blood cultures.
CT scanning identified inflammatory changes within the left greater sciatic foramen, involving the piriformis and gluteus medius muscles, along with a thickened ectatic dural sac extending into the spinal canal suggestive of meningeal inflammation. Subsequent MRI revealed a large intraspinal cystic structure.
Intravenous antibiotic therapy was instigated and an image-guided aspiration performed. Frank pus was drained and E. Coli cultured. Appropriate antibiotics were continued and her symptoms resolved.
Conclusion
Dural ectasia is a common structural feature of Marfan syndrome and under ultrasound visualisation would be indistinguishable from hydrosalpinx.
In reflection of this case our team felt that in cases of connective tissue disease or possible skeletal abnormalities that further imaging must take place prior to ultrasound guided gynaecological procedures to eliminate structural differences.
Inadvertent piercing of dural ectasia is a rare cause of spinal abscess.

P102- Spontaneous resolution of paediatric hydrosalpinx: a case for conservative management

Zainab Kazmi[1,2*], Sujata Gupta[2], Mike Dobson[2]
[1]University of Manchester, Manchester, UK; [2]Lancashire Teaching Hospitals Trust, Preston, Lancashire, UK

Background
Hydrosalpinx is a rare cause of abdominal pain in non-sexually active paediatric and adolescent patients, although there are cases documented in the literature. Diagnosis of this presentation can made via ultrasound scan.
Case
A 12-year-old non-sexually active girl presented to the surgical admissions unit with left-sided acute abdominal pain that settled within 24 hours of admission. An ultrasound scan performed by a sonographer suggested the presence of a hydrosalpinx.
A post-discharge follow-up appointment with a consultant paediatric and adolescent gynaecologist demonstrated no symptomology but a repeated ultrasound scan by a second sonographer more clearly revealed continued presence of hydrosalpinx, which had now grown larger.
A decision to operate was made on the principle of risk minimization. The risk of future ovarian torsion is increased in hydrosalpinx, so a decision to operate was made to preserve fertility even in the absence of any troublesome physical symptoms.
Prior to the operation, an MRI was performed to confirm the site of the hydrosalpinx. However, the MRI revealed no tubal masses, suggesting spontaneously resolved hydrosalpinx. A consultant-administered ultrasound scan confirmed no present tubal abnormalities.
Conclusion
This case demonstrates the possibility of spontaneous resolution of paediatric hydrosalpinx demonstrated by repeat imaging, which has not been previously reported. Our recommendation now, based on the same principle of risk minimization mentioned above, is for conservative management of asymptomatic paediatric hydrosalpinx shown on ultrasound, alongside education of the patient and parent/guardian of the presentation of tubal torsion, for which emergency admission and surgery would then be indicated.

P103- Subfertility and Severe Endometriosis: Is endoscopic surgical intervention now the way forward?

Rizwana Yakub[1*], Sandu-Aana Lakshmi[1], Sara Nasser[1], Gaity Ahmad[1], Peter Byrne[1], Zul Anjum[1]
[1]*Royal Oldham Hospital, Oldham, Manchester, UK*

Endometriosis is a leading cause of pelvic pain and subfertility, with a 20-30% incidence in infertile patients. The presence of severe endometriosis (stage III/IV) is widely accepted as negatively impacting fecundity; with rates of conception being reported at almost 0. Whilst gynaecologists are fully aware of this, the decision of how to primarily manage subfertility in this scenario is highly debatable especially in the absence of any other endometriosis related symptoms. There remains a divide with assisted reproduction specialists championing IVF/ICSI as the primary treatment of choice and laparoscopic gynaecologists supporting excision of endometriosis +/- subsequently combining it with an assisted reproductive approach.
In this review we aim to evaluate the conflicting literary evidence, to provide a summary which can help clinicians when counselling patients of the best primary approach for severe endometriosis related sub fertility. Currently there are no published, large, well designed, randomised control trials or meta-analyses comparing the efficacy of all three approaches.
The majority of evidence recognises assisted reproduction as an effective treatment modality which has favourable clinical pregnancy rates, ranging from 32% - 60%. Studies reviewing endoscopic surgery though limited suggest pregnancy rates of 54%. Furthermore when subsequently combined with IVF/ICSI achieving cumulative clinical pregnancy rates of 56%-75%, consistently higher than that achieved by surgery alone or IVF alone in these studies. With evidence also suggesting that the optimal time to conceive is <2 years after surgery by IVF/ICSI and will achieve 2.65 times the pregnancy rate that IVF alone will achieve.

P104- Successful Novasure endometrial ablation following laparoscopic suturing of uterine perforation

Susan Tracey[1*], Olorunfunmi Odusoga[1], Andrew Pickersgill[1], Ciaran Clarke[2]
[1]*Stepping Hill Hospital, Stockport, UK;* [2]*University of Manchester, Manchester, UK*

We present a 26 year old para 2 patient with a two year history of dysfunctional uterine bleeding. Management with tranexamic acid, norethisterone and Mirena IUS had failed. Ultrasonography and bloods were normal. After counselling about further management, as she had completed her family she opted to have Novasure ablation for her dysfunctional bleeding and laparoscopic bilateral salpingectomy for permanent contraception.
Following dilatation of the cervix at surgery and insertion of the Novasure device, the device opened to 3.5cm cavity width with no suggestion of perforation; however the cavity integrity assessment failed twice. The procedure was abandoned; we then proceeded with laparoscopy where a small perforation to the uterine fundus was noted. The perforation was closed laparoscopically with two interrupted sutures and bilateral salpingectomy was performed.
A subsequent cavity integrity assessment was successful and Novasure ablation was performed under direct vision.
Conclusion
The Novasure system uses carbon dioxide to verify cavity integrity prior to performing the procedure. Failure of this cavity integrity assessment should raise suspicion of uterine perforation.
As the Novasure is an expensive single use device, our case highlights the options available in conserving resources in cases where uterine perforation occurs and allows the patient to still benefit from an effective day case procedure. This case brings to the fore the value of laparoscopic suturing in gynaecological surgery.
Opting for laparoscopic salpingectomies meant our patient could potentially reduce her risk of ovarian cancer in future, prevent post ablation sterilisation syndrome and achieve permanent contraception.

P105- Surgical Approach for the Benign Ovarian cysts at York Teaching hospital NHS Foundation Trust

Georgios Grigoriadis[1*], Charilaos Charalampidis[1], Fawzia Sanaullah[1]
[1]*York Teaching Hospital NHS Foundation Trust, York, UK*

Laparoscopic management of ovarian cyst is well established offering distinct advantages of quick postoperative recovery and reduced cost. Operative laparoscopy should be our primary surgical approach to treat majority of benign ovarian cysts.
Objective:
To assess the surgical approach for the management of benign ovarian cyst at York Teaching Hospital Hospital.
Design:
Retrospective cohort study
Method:
All consecutive women who were operated for ovarian cyst not suspected to be malignant from Sept 2013 to Sept 2014. Total 61 women were identified from the theatre computer record and pathology department. The data was collected from the hospital computer.
Results:
Laparoscopic approach was used in 43 (71%) and 18 (29%) had laparotomy. The mean operation time in the laparoscopy group was 100 minutes (range 40 to 237min) compared to 96 minutes (range 51 to 270 minutes) in the laparotomy group. Mean cyst diameter was 10cm (2 to 31cm) with one 26cm cyst managed laparoscopically. 31 (51%) were managed by cystectomy and 30 (49%) had oophorectomy preferably in women over 45years. In

the laparoscopy group, 43 women were discharged on the same day or following day compared to average 3.5 days in laparotomy group. Two women required second operation by oncologist to complete the operation as the histology suggested borderline and adenocarcinoma. There were no major complications in both groups.

Conclusion:

Laparoscopy should replace laparotomy in the management of benign ovarian cyst.

Further audit is needed in the department to assess the factors affecting the surgical approach.

P106- SURGICAL MANAGEMENT OF OVARIAN CYST IN PREMENOPAUSAL WOMEN

Subashini Sivalingam[1*], Hannah Wigginton[1], Zeiad El-Gizawy[1]
[1]*Royal Stoke Hospital, Stoke-On-Trent, UK*

Aim: To assess our surgical management of premenopausal women with Ovarian cysts.

Method: All Women who underwent surgery for ovarian cyst were identified from the surgical database in a 12 month period (August 2013 to July 2014). A total of 100 women were audited. BSGE/RCOG guidance was used as the auditable standard.

Results: Cysts were managed by laparoscopic surgery in 86% and laparotomy in 14% (4 at caesarean section). Method of surgery was ovarian cystectomy in 52% (45 laparoscopic and 7 open), aspiration in 10%, oophorectomy in 36% and oophorectomy with hysterectomy in 2%.With laparoscopic approach the cyst was removed intact 63%, spill of cystic contents were noted in 37%. However in all the cases the specimen was removed in a contained bag. During open surgery this was 57% and 43% respectively. 50% of the women had midline and lower transverse laparotomies each. There were no significant complications noted. In this cohort 4% women had borderline malignant tumours. Among the 10 open surgery for ovarian cyst/mass 7 benign and 4 were malignant tumours.

The size of the cyst at the time of booking for surgery was <5cm in 36%, 5-7cm in 29% and >7cm in 31%. In 7% this was not available. In our study group only 8 women (13%) <40 years had all the recommended tumour markers 32 women (52%) had atleast 1 other tumour marker.

Recommendation: Aspiration of ovarian cysts should be avoided as this increases recurrence. Women with cyst <5cm should be offered conservative management.

P107- Survey on Laparoscopic Specimen Retrieval among Consultant Gynaecologists in UK

Sridevi Sankaran[1*], Funlayo Odejinmi[2]
[1]*Frimley Health NHS Foundation Trust Frimley UK;* [2]*Whipps Cross University Hospital Leytonstone UK*

As laparoscopic surgery involves major surgery through small incisions specimen retrieval can present a challenge, for cosmesis, ease of specimen delivery, pain and potential complications. We thus conducted a survey of consultant laparoscopic surgeon members of the BSGE to determine practices of retrieval of specimen after laparoscopic surgery and the reasons behind the choice of ports sites for retrieval for common laparoscopic procedures

Results

Of the 460 registered consultants 187 (40%) responded to the SurveyMonkeyÔ questionnaire. The most commonly used port for specimen retrieval was umbilical 10mm ports for ectopic pregnancy, ovarian cysts and endometriomas 49%,43% and 43% respectively with no extra port used or existing port extended just for retrieval of specimen.

The second commonest port was suprapubic 10mm ports 35% for ectopic pregnancy,34% for ovarian cysts and 36% for endometriomas.

During the retrieval of specimen most consultants routinely use a retrieval bag.

For laparoscopic myomectomy 84 % of those who performed the procedure would retrieve the specimen by power morcellation, as opposed to a minilaparotomy or posterior colpotomy, 5% of them would perform morcellation in a bag. For laparoscopic subtotal hysterectomy the figure was 93%.

Conclusion

Umbilical port is the most commonly used port for specimen retrieval among UK gynaecologists, most would not insert an extra port just for specimen retrieval. For more complicated procedures despite the recent scare most will still use power morcellation for laparoscopic myomectomy and subtotal hysterectomy.

P108- Temporary psychogenic lower limb paralysis (conversion disorder) following diagnostic laparoscopy for investigation of pelvic pain

Sami Shawer[1*], Atul Nalawade[1], Asokan Krishnan[1], Nicholas Matthews[1], Ajay Asokan[1]
[1]*North Tees and Hartlepool Foundation Trust Stockton-On-Tees UK*

Background:

Functional (psychogenic) loss or weakness of motor functions following hospital admission or surgery is a known conversion disorder. Formerly known as "hysteria", conversion disorder is a rare complication after gynaecological laparoscopies. Patients with conversion disorder tend to regain their sensorimotor functions spontaneously. This is the second case reported with similar complication post-laparoscopy and the first case reported after diagnostic laparoscopy.

Case:

A 25 years old female patient underwent a day case diagnostic laparoscopy under general anaesthesia to investigate pelvic pain. No intra-operative complications were noted. Patient was otherwise fit and healthy. During immediate recovery period, she developed unexplained bilateral lower extremity paralysis. MDT approach was adopted and patient was reviewed by neurology and orthopaedics teams. Diagnostic tests including MRI to exclude any organic aetiology, were all normal. After exclusion of possible organic causes, diagnosis of conversion disorder was made. Patient spontaneously regained full sensorimotor functions around 36 hours after the surgery. Patient was discharged 48 hours post-operative without neurological complication.

Conclusion:

Conversion disorder causes patients to suffer from neurological symptoms, such as numbness, blindness, paralysis, or fits without organic cause. It is thought that symptoms arise in response to stressful situations affecting patient's mental health. The loss of sensorimotor functions as part of conversion syndrome is rare, but still a possible post-operative complication which may be encountered with gynaecological laparoscopies. MDT approach and exclusion of other organic lesions is crucial to establish this diagnosis.

Reference(s):

1.Berhane L, Kurman R, Smith S. Lower extremity paralysis after operative laparoscopy from conversion disorder.A case report.J Reprod.Med1998;43:831-5.(and other references).

P109- The birth of an endoscopic audit tool in a developing country

Raj Dodia[1*], Timona Obura[2]
[1]*University of Oxford Oxford UK;* [2]*Aga Khan University Hospital Nairobi Kenya*

PURPOSE: Clinical audit determines gaps between what is and what should be done, revising any ‘lacks' in patient care processes, and is a vital step in quality improvement strategies including accreditation processes.

DESIGN AND METHOD: Laparoscopy and hysteroscopy procedures done at the Aga Khan University Hospital Nairobi in 2013 were audited against a newly designed endoscopic audit tool. Laparoscopy procedures were coded as diagnostic, for ectopic pregnancies, myomectomies, endometriosis, cystectomies, hysterectomies, and other (specify). Hysteroscopies were coded as diagnostic, polyp resection, myoma resection, adhesiolysis, ablation, and other (specify). Possible complications included injury to intraperitoneal and retroperitoneal organs, herniae, pneumoperitoneum related, nerve injury, venous thromboembolism, death, wound infections, and functional compromise after surgery. Electrosurgical sources (monopolar, bipolar, ultrasound) utilized and procedure duration was noted. Hysteroscopic distention fluid was identified and the end deficit recorded.
RESULTS: In 2013, there were 192 laparoscopies and 193 hysteroscopies. Of 286 patient records, 78.3% did not have complete data as per the designed form (except complications). Only 51.6% of the complete data records were by departmental faculty.
LIMITATIONS: Errors in data transcription; incomplete recording of procedure notes.
CONCLUSION AND COMMENTS: The inclusion of this data tool as a necessary and compulsory part of the operation notes for every endoscopic procedure may assist in improving procedure documentation and can provide (e.g. six monthly, or yearly) a valuable resource for audit, which would ultimately lead to higher quality of patient care once the clinical audit cycle is followed through, including feedback to groups as well as individuals.

P110- The impact of 3D Einstein vision laparoscopic technology on Theatre efficiency with surgeons and theatre staff perspective

Oudai ALI[1*]
[1]*West Cumberland Hospital Whitehaven UK*

Objective
The benefit of the 3D vision in minimal access surgery is well recognised through incorporation in the DaVinci system. However the 3D concept in standard laparoscopy needs to be evaluated and its value demonstrated in the actual theatre environment.
Methods
The 3D system was tried over 4 weeks in laparoscopic gynaecology procedures. Surgeons and theatre staff feedback was collected. Operating time was observed for TLH. The time to close the vault laparoscopically was considered a function of the improved vision and video clips of suturing with 2D and 3D were compared.
Results
There was positive feedback supporting many aspects particularly superior image quality,feasibility and enhanced surgical experience with shorter operating times.For one surgeon's standard technique in performing TLH with intracorporeal suturing the vaginal cuff, the average operative time dropped to 74.17(SD;13.75)mins from 127mins(SD;+/-14.4) and the vault closure to 11.21min(SD;+/-1.85) from 20.82(SD;+/-3.16)min. The unique feature of autonomous warming of the scope was noted to keep the view stable avoiding the frequent interruptions to defog. The sterile sleeve over the scope concept was safe and easy to use and kept the scope ready for the next cases.
Conclusion
The introduction of approved innovations to theatre systems should be tried in a controlled structure where safety, acceptability and impact on productivity are tested and feedback is analysed. Minimal access surgery adopted many developments across its history however the best investment will always be the continuous improvements in optics. 3D vision improved the surgical performance and the increased precision translates into better efficiency.

P111- The Impact of the Presence of a Colorectal Surgeon on the Management and Outcomes of Surgery for Rectal Endometriosis

Jonathan Clarke[1,2*], Neda Taghinejadi[2], Charles Craddock[2], Thomas Holland[2], Richard Cohen[2], Ertan Saridogan[2], Alfred Cutner[2]
[1]*Imperial College London London UK;* [2]*University College London Hospitals NHS Foundation Trust London UK*

For women with extensive endometriosis involving the bowel, multidisciplinary management including a colorectal surgical opinion has become the standard of care. This study examines how the presence of a colorectal surgeon influences the surgical management and post-operative colorectal symptomatology of rectal endometriosis.
The BSGE Endometriosis Centre Database was examined for patients with rectal endometriosis. The presence or absence of a colorectal surgeon upon intraoperative techniques used and patient self-assessment of symptoms, quality of life and effect on daily activities following surgery was examined. Fisher's Exact Test was used to compare treatments and symptomatology with and without a colorectal surgeon present.
426 patients were present in the database, of which 106 (24.9%) had rectal involvement. Teams involving a colorectal surgeon were significantly more likely to perform surgery to the bowel (97.5% vs. 83.3%, $p<0.05$). Scissors were significantly more likely to be used in the presence of a colorectal surgeon (52.4% vs. 21.2%, $p<0.05$) while bipolar diathermy was significantly less likely to be used (4.8% vs. 19.4%, $p<0.05$). There is no significant difference in the patients' symptoms or quality of life between groups following treatment.
The presence of a colorectal surgeon in rectal endometriosis surgery significantly alters surgical treatment, however the ultimate impact of these differences on a patient's overall outcomes remains unclear. It is probable that colorectal surgical involvement is reserved for more extensive cases of rectal endometriosis, and the use of scissors may reflect a need to avoid administration of electrosurgical energy in proximity to the bowel.

P112- The learning curve: do the first few laparoscopic hysterectomies carry a higher risk of complications?

Annabel Grossmith[1*], Aarti Shetty[1], Jemma Mistry[1], Fariha Rahim[1], Sujata Gupta[1]
[1]*Lancashire Teaching Hospitals NHS Foundation Trust Preston UK*

The increasing trend towards choice of laparoscopic route for hysterectomy in our unit, has led to an expansion in personnel competent in benign gynaecological endoscopy. We conducted an audit of practice at a large teaching hospital to investigate our results for laparoscopic hysterectomies. All patients coded as having had a hysterectomy performed over a three year period between 1/6/11 and 31/5/14 were identified retrospectively from prospectively recorded computerised theatre data. For each case, the indication for hysterectomy, surgical approach, duration of surgery, estimated intra-operative blood loss, duration of stay, complications, need for conversion to open approach, BMI, and previous abdominal surgery were ascertained from computerised casenotes and theatre logs. 1056 patients were identified. Of these, 253 had laparoscopic hysterectomies. The level of experience of the surgeon was noted, and the above data was compared for each successive year during the period of investigation to assess the learning curve within the department. The findings and conclusions will be discussed together with our suggestions for future local policy.

P113- The outcome of laparoscopic assisted vaginal hysterectomy in a district hospital

Neelam Agrawal[1*], Shamma Al-Inizi[1]
[1]*South Tyneside hospital South Shield UK*

Vaginal route is the recommended route for benign indications of hysterectomy. Though laparoscopic assisted vaginal hysterectomy could replace abdominal route where early stage 1 endometrial carcinoma, enlarged uterus, advanced endometriosis and no uterine descent.
We reviewed case-notes for 38 patients who underwent this procedure at STH over 2 year period to check conversion rate, intra-operative and postoperative complication rates, hospital stay, readmission and reoperation rates. About 85% procedures were completed by laparoscopy and conversion rate was 15.7%. Among them 5 patients (11.3%) has had previous abdominal surgery. Half of them (44.7%) underwent peritoneal washing in addition to LAVH+BSO for endometrial carcinoma. Intra-operatively 5 patients (11.3%) bled > 900 ml. The major intra-operative and postoperative complication rate was 2.6% each. None of them has had trauma to bladder, bowel or ureters. No significant differences were seen in intra-operative and postoperative complication rates of patients who were morbidly obese (BMI>35), enlarged uterus (14 weeks) or who underwent additional procedures (bilateral salpingo-oophorectomy, adhesiolysis or prolapse surgery). Majority of them (40%) went home day 2 and only 3 patients (7.9%) needed to stay more than 3 days. None of them was readmitted secondary to surgery complications and reoperation rate was 2.6%. None of the variables studied: age, medical problem, morbidly obesity, enlarged uterus, additional procedures were found to have any association with readmission or reoperation. Conclusion: Laparoscopic assisted vaginal hysterectomy can be performed successfully in most patients with benign indications and early stage of endometrial carcinoma with low complication rate and short hospital stay.

P114- The relationship between pelvic vein incompetence and chronic pelvic pain in women: an evidence synthesis

Jane Daniels[1*], Rita Champaneria[1], Laila Shah[1], Janesh Gupta[1], Jon Moss[2], Judy Birch[3], Lee Middleton[1]
[1]University of Birmingham Birmingham UK; [2]North Glasgow University Hospital Glasgow UK; [3]Pelvic Pain Support Network Poole UK

Background
Pelvic congestion syndrome (PCS) is described as chronic pelvic pain (CPP) arising from dilated and refluxing pelvic veins, although the causal relationship between pelvic vein incompetence (PVI) and CPP is not established. Percutaneous embolisation is the principal treatment for PCS, with high success rates often cited.
Objectives
To systematically review the association between PVI and CPP and the effectiveness of embolisation for PVI.
Methods
A comprehensive search strategy encompassing various terms for pelvic congestion, pain and embolisation was deployed in 17 bibliographic databases. There was no restriction on study design. Methodological quality was assessed using appropriate tools. The quality and heterogeneity generally precluded meta-analysis so results were described narratively.
Results
We identified six association studies and 21 case series and one poor quality randomised trial of embolisation.
We found the associations between CPP and PVI were generally fairly similar, with three of five studies with sufficient data showing statistically significant associations (odds ratios between 31 and 117). The prevalence of PVI ranged widely, although the majority of women with PVI had CPP. Early substantial relief from pain symptoms was observed in approximately 75% of women undergoing embolisation, which generally increased over time and was sustained.
Conclusions
The quality of data supporting the treatment of PCS is limited and of variable methodological quality. There is some evidence to tentatively support a causative association, but it cannot be categorically stated that PVI is the cause of CPP in women with no other pathology. Embolisation appears to provide symptomatic relief in the majority of women and is safe.

P115- To morcelate or not to morcelate : a case series of 'abnormal' fibroids

John Wahba[1,2*], Tom Setchell[3], James Richard Smith[2], Tariq Miskry[3], Alan Farthing[1,2]
[1]Imperial College London London UK; [2]Queen Charlotte's & Chelsea Hospital, Imperial College Healthcare NHS Trust London UK; [3]St. Mary's Hospital, Imperial College Healthcare NHS Trust London UK

The issues surrounding the use of power morcellation during laparoscopic myomectomy for symptomatic fibroids have caused widespread controversy amongst gynaecologists. Such issues include the risk of severe trauma and seeding of morcellated tissue in the abdominal cavity. One of the most serious concerns is the risk of disseminating occult malignancy such as an inadvertent leiomyosarcoma (LMS); a risk which the FDA has quoted as 1:350 cases. However, the number may be as low as 1:7400 according to research. The incidence of finding 'unexpected' pathology is 1.2% according to one study. Evidently, not all 'unexpected' pathologies will carry a serious prognosis, but distant spread would clearly negatively affect those with LMS.
We present a case series of pre-menopausal women who presented with symptomatic fibroids for which they underwent a myomectomy or a total abdominal hysterectomy. Histological and radiological examination later revealed unexpected pathologies such as LMS and widespread endometrial sarcoma. In two cases, laparoscopic myomectomy was converted to open surgery due to the intra-operative findings and abnormal dissection planes). In both cases power morcellation was not utilised due to open conversion. Following subsequent hysterectomy one patient was shown histologically to have had complete tumour excision the original open myomectomy. The other patient was unfortunately found to have disseminated extra-uterine pelvic spread.
It is unclear whether the risk of dissemination of inadvertent LMS differs with power morcellation versus myomectomy without morcellation (e.g. open myomectomy). Understandably, any disruption of malignant tissue intra-operatively may have significantly poor consequences in those with a sarcoma.

P116- Torted Haematosalpinx: A pain beyond compare

Lewis Nancarrow[1*], Tarun Ghosh[1]
[1]Nobles Hospital Isle of Man Isle of Man

Torted haematosalpinx is a rare condition with the majority of cases associated with an ovarian torsion; however an isolated torted fallopian tube is very rare with only a few cases being reported in the literature.
We present a case of a 41 year old nulliparous women who attended with severe left iliac fossa pain that had been getting progressively worse over a 2 week period. On examination abdomen was soft with minimal tenderness in left iliac fossa with no rebound or guarding. Bloods and inflammatory markers were normal. She was known to have hydrosalpinx previously but on admission ultrasound there was a haemorrhagic left ovarian cyst.
Patient was taken for Laparoscopy and findings showed a grossly enlarged torted left haemosalpinx. The left fallopian tube had twisted 4 times on itself. The right tube showed hydrosalpinx and otherwise the pelvic anatomy was grossly normal. The patient underwent bilateral salpingectomy and on histology both tubes showed evidence of chronic salpingitis and the left tube showed haemorrhagic infarction. Vaginal swabs were negative and no cause was evident for the salpingitis. Postoperatively the patient had near complete resolution of her pain.

This is a rare cause of sudden onset abdominal pain but should be considered as a potential differential. On review of the literature and from our own scans, imaging for tube torsion has a low sensitivity therefore early laparoscopy would seem to be the most appropriate method of diagnosis in patients presenting with these symptoms and no definitive diagnosis.

P117- Total Laparoscopic hysterectomy (TLH): a Holly Grail or a Harbinger of Trouble?

Karina Datsun[1*], Khaled Elsapagh[1], Mohammed Allam[1]
[1]NHS Lanarkshire Wishaw UK

Objective. The aim is to analyze indications, outcomes, preoperative and postoperative histopathology results, compare the intraoperative and short-term postoperative complications of laparoscopic hysterectomy and total abdominal hysterectomy and to analyze the shift towards laparoscopic approach in a district general hospital (NHS Lanarkshire).
Methods. Retrospective analysis of 250 cases: 166 cases of total abdominal and 84 cases of total laparoscopic hysterectomies.
Results. Over a period of 16 months we performed 250 total hysterectomies.
2/3 of them (n=166, 66.4%) were TAH and remaining 1/3 (n=84, 33.6%) TLH.
To compare, only 21 TLH were performed over a period of 12 months after introduction of total laparoscopic hysterectomy in our department in 2012.
There were total 3 cases of bowel injury: 1 case during TLH, which required conversion to laparotomy and 2 cases during TAH.
There were 2 cases of bladder injury in patients who underwent TAH; none of the TLH was complicated by bladder or ureteric injury.
There was only 1 re-admission to the hospital after TLH versus 19 re-admissions post TAH.
Majority of the pre-operative histopathology reports were confirmed postoperatively. However, there were nine cases of undiagnosed cancer.
Conclusion. Laparoscopic hysterectomy can be safely done with a low and reasonable complication rate, and a shorter hospital stay.
As experience is gained the operation time, complication rate and hospital stay are decreased.
It is possible to change a practice within settings of a district general hospital. The skills of preforming TLH are reproducible and most gynaecologists can adapt the technique for laparoscopic hysterectomy.

P118- Total Laparoscopic Hysterectomy: incorporating the findings of a retrospective audit for designing an Enhanced Recovery Care Pathway at a tertiary London teaching Hospital

Tina Kapoor[1*], Kim Lawson[1], Amer Raza[1]
[1]Chelsea and Westminster Hospital NHS Foundation Trust London UK

Objective: The primary objective of the study was to obtain updated surveillance statistics for laparoscopic hysterectomy procedures in order to lay a foundation for designing an enhanced recovery care pathway in our unit.
Methodology: A Retrospective case notes review was taken of all women who underwent Laparoscopic Hysterectomy between May 2012 and May 2014. Demographic data was collected and entered onto MS Excel spreadsheet. Details of surgical procedure including indication, type of surgery, duration of procedure, any intra-operative and/ or post-operative complications and duration of hospital stay were entered electronically for ease of analysis. Any adverse events including unplanned admissions following discharge were recorded.
Results: A total of 38 hysterectomies were performed over these 2 years of which 56 % were performed for benign indications. 84% were completed laparoscopically thus giving a conversion rate of 12.5%. Mean operating time was 199 minutes. 32% of patients were discharged within 48 hours with a mean in-patient stay of 63 hours. Main reasons delaying discharge were postoperative nausea, vomiting, pain, and urinary retention. There were no readmissions following the surgery during this period.
Conclusion: This analysis was crucial to devise a Care Pathway that would ensure patient safety while delivering a high standard of care. With appropriate multi-disciplinary team input and a robust enhanced recovery programme, advanced laparoscopic surgery can be safely performed with improved patient turnover within 36-48 hours, while keeping complications rates at the minimum.
A comprehensive literature review has been undertaken and is presented along with.

P119- Training new hysteroscopists in the setting of a new out-patient hysteroscopy service

Fani Gkrozou[1*], Eve Gaughan[1], Bronwyn Middleton[2], Richard Pyper[1], Natasha Waters[1]
[1]Southlands Hospital West Sussex UK; [2]St Richard's Hospital West Sussex UK

Introduction: The development of ambulatory hysteroscopy requires outpatient procedures to be performed by an experienced clinician. The service requires the clinician not only be able to perform a range of diagnostic and operative procedures but also to perform appropriate risk assessment for each individual patient and modify these technique for difficult cases on a patient who is awake. It is important to establish safe protocols for teaching new hysteroscopists in this setting.
Results: Our 6/12 audit of the out-patient hysteroscopy service showed that hysteroscopy and biopsy could be performed in 90% of women referred with a history of unsuccessful or inadequate pipelle endometrial biopsy.
No perforations in over 1200 OPH procedures performed in our trust. In 40% of cases vaginoscopic approach was used. It took trainees about 48 procedures to learn all techniques including vaginoscopy with both flexible and rigid hysteroscopes. 7% required cervical dilatation and 1% required dilatation under direct vision with micro-hysteroscopy using graspers and scissors. 4% of patient felt sick and 1% had vaso-vagal episodes. 5% of procedures could not be completed.
Conclusion: Although it is possible to shorten pre-clinical training by attending hands on courses, the attainment of skills for outpatient hysteroscopy is individual and competence based. A simple check list would help to guide a new hysteroscopist and will ensure patient safety, while being directly supervised by an experienced clinician

P120-Treating symptomatic uterine fibroids with myomectomy: Current views and practices of consultants

Fusun Sirkeci,[1,2*] Isaac Manyonda[1,2]
[1]St George´s University of London London UK; [2]St George´s Healthcare NHS Foundation Trust London UK

With women increasingly postponing childbirth into their late thirties and early forties, when fibroids are more numerous and more symptomatic, the number of women seeking uterus-sparing treatments is increasing. With rising education and empowerment, women now demand a wider range of treatments that not only meet their individual needs, but also have an evidence base. Since myomectomy arguably remains the most commonly used treatment, we sought to establish current views and practices concerning myomectomy among consultant gynaecologists in the UK.
Methods: We constructed a 25-stem questionnaire that addressed issues such as the pre-myomectomy use of GnRh analogues (GnRHa) and ulipristal acetate, routine cross-matching of blood, use of cell salvage, any limitations on number of fibroids to be removed at open, laparoscopic and hysteroscopic myomectomy, intraoperative approaches to reduce blood loss etc. This was then emailed to xxx consultant gynaecologists.

Results: To date responses have been obtained from 280 consultants, a response rate of 13%. Open myomectomy is the most common route, with 80% surgeons using GnRHa despite 60% believing that GnRHa destroy tissue planes. Vasopressin is the most common intervention to reduce intraoperative blood loss. The majority of respondents (62%) were not influenced by the size of the fibroids.
Conclusions: We acknowledge the low response rate and therefore interpret our findings with caution. There does not appear to be any major changes in gynaecologists' views and practices regarding myomectomy in recent years, including with regard to the use of GnRHa.

P121- Treatment of caesarean scar ectopic pregnancy at the Royal London Hospital- a series of 19 cases and a literature review

Natalie Cooper[1,2*], Mary Gbegbaje[2], Elizabeth Ball[2], Emeka Okaro[2]
[1]*Blizard Institute, Queen Mary University London UK;* [2]*Royal London Hospital London UK*

Caesarean scar pregnancy is rare which means that the treatment options are difficult to evaluate. We present a series of 19 cases of caesarean scar pregnancy, treated at the Royal London Hospital between September 2007 and October 2014. During this time frame our treatment evolved so that the current management combines the use of methotrexate, suction curettage and cervical cerclage. None of the cases experienced any complications.
We also present the results of a literature review examining the reported methods for treating scar pregnancy, including surgical and medical methods and uterine artery embolization.

P122- Ultrasound, Intra-operative and Histological correlation of ovarian cysts in pre-menopausal women

Ilias Nikolopoulos[1*], Pinky Khatri[1], Graham Phillips[1]
[1]*James Cook University Hospital Middlesbrough UK*

Preoperative differentiation between the benign and the malignant ovarian cysts is useful in planning their management. Pelvic ultrasound is the most effective way to assess these cysts. The standard treatment for benign cysts is laparoscopic ovarian cystectomy.
Objective:
To evaluate pre operative accuracy of ultrasound assessment of ovarian cysts with laparoscopic findings and histological confirmation.
Methods:
Retrospective analysis of 29 cases of laparoscopic ovarian cystectomies was done. Ultrasound scan, laparoscopic and histological results were compared.
Results:
Preoperative assessment of all the cysts indicated a benign nature resulting in laparoscopic management. Laparoscopic findings correlated with USS in 25/29 (86%) cases resulting in cystectomies. In the remaining 4 cases, intraoperative findings suggested complex nature necessitating oopherectomy and peritoneal cytology.
Histological examination proved that all the cysts were benign. The histology included serous/mucinous cystadenoma, dermoid, endometrioma, luteinised corpus luteal cysts and simple cyst. This showed 100% correlation with USS findings.

P123- Uterine Adhesions: An Iatrogenic Cause of Chronic Pelvic Pain

Rebecca Mallick[1*], James English[1]
[1]*Royal Sussex County Hospital Brighton UK*

Over the past 10 years there has been increasing debate over the closure versus non-closure of peritoneal layers at the time of caesarean section (CS). Historically closure of both layers was advised as this was thought to correctly restore anatomy and reduce the risk of adhesion formation. Current NICE guidance advises against the closure of the peritoneal layers and this now appears to be common practice amongst obstetricians nationwide.
Uterine to abdominal wall adhesions are not a well documented long term complication associated with CS, however with this change in practice, we have increasingly encountered extensive uterine adhesions at the time of laparoscopy in women presenting with pelvic pain with approximately 20 cases in the past 10 years. Although these adhesions cannot definitely be linked to surgical practice it seems credible that the practice of not closing the peritoneum at CS may be a significant contributing factor.
We present one of these cases, resulting in chronic pelvic pain, where extensive uterine to abdominal wall adhesions were found at the time of laparoscopy and adhesiolysis was performed using a harmonic scalpel prior to a total laparoscopic hysterectomy. We also review the literature regarding closure versus non-closure of the peritoneal layers and explore possible long-term surgical implications.

P124- Uterine Artery Embolisation for symptomatic fibroid uterus

Sreebha Rajesh[1*], David Shaw[2], Chris Kremer[2]
[1]*QA Hospital Portsmouth UK;* [1]*Midyorks Hospital NHS Wakefield UK*

Aim: Determine outcome for women with symptomatic fibroids treated with Uterine Artery Embolisation. Audit the adherance to NICE guidelines: UAE for fibroids.
Materials and methods: A retrospective audit of 54 patients who underwent UAE from 2007-2013.
Results: Steady increase in patients accepting UAE for fibroids over the years with highest number of 42%(23/45) in 2013. The age group was 28-52 years. Out of the 8 nulliparous women, one had a successful pregnancy after a year and another ended with emergency hysterectomy for sepsis.. The commonest presenting symptoms were menorrhagia (63%) and pressure symptoms (14%). More than 75% of patients had UAE within 6 months of referral by a gynaecologist. Day case UAE was offered to patients from December 2011 and so far 80% had successful bilateral UAE as day case. The complication rates were immediate-3.8%, early-1.9% and late-9.6%. 3 patients underwent repeat UAE and 4 ended up with a hysterectomy. Out of the 73% of patients who attended GOPD follow up post procedure; 80% were satisfied with results and 91% had MRI evidence of shrinkage in fibroid size.
Conclusion: UAE is widely accepted treatment for symptomatic fibroids. More and more UAE's have been performed as day case since 2012 with a low re-admission rate (11.5%). Re-intervention rate was comparable to literature.
Recommendation: Ensure UAE leaflets are offered to patients and copy of discharge letter post-UAE are sent to the gynaecologist so that patients are followed up in the GOPD post procedure.

P125- Variables which Influence the Duration of Laparoscopic Hysterectomy

Sarah Manohar[1*], Sujata Gupta[1]
[1]*Royal Preston Hospital Preston Lancashire UK*

INTRODUCTION: There are many factors which affect the Duration of laparoscopic Hysterectomies but it is not known which of these factors influence the duration of surgery. The purpose of this study is to analyse the effect of the size of the uterus, previous surgeries and Body mass Index on the duration of 69 laparoscopic hysterectomies performed by one consultant over a period of 2012-2015.
RESULTS: There was a statistically significant overall reduction in the duration of surgery performed by the consultant over the 3.5yr study (p=

0.00642). Factors such as BMI and uterine size also increased the length of surgery although this was not found to be statistically significant. There was only one significant complication in this case series.

CONCLUSION: Laparoscopic hysterectomy is an effective way of performing hysterectomy with minimal complications. There is a positive correlation between the duration of surgery and the uterine size. But perhaps the most important factor is surgeon's experience on its own. Certainly, this is what our case series reflects.

P126- Visual Numeric Endometriosis Scoring system, VNESS. A new endometriosis scoring system aimed to facilitate communication and documentation of disease severity and surgical findings

Shaheen Khazali[1,2*]
[1]*CEMIG-Centre for Endometriosis and Minimally Invasive Gynaecology Chertsey UK;* [2]*Ashford and St. Peter's Hospitals NHS Foundation Trust Chertsey UK*

Since 1918, at least 17 systems have been proposed for staging, categorization or description of endometriosis. Despite multiple attempts, an easy to use, reproducible, clinically useful and properly validated system is yet to be developed.

The Visual Numeric Endometriosis Scoring System (VNESS) is an attempt to address the shortcomings of previously proposed systems.

VNESS consists of 8 numbers, each between 0-4, representing the severity of endometriosis in each anatomical location in the pelvis, starting from left adnexa, going down to pelvic sidewall, then to the uterosacral complex, then to the uterovesical fold and pouch of Douglas and back up the right side. The intention is that one can easily picture how severe the disease was in different compartments. Examples are provided within the scoring sheet to assist with scoring and a comment box is provided to describe disease outside the pelvis or to provide further description. For example: 313/04/302 – small nodule on terminal ileum.

This presentation reports on the first phase of this project: conceptualization and consultation with experts. Phase two includes two separate studies to assess the inter-rater and intra-rater validity using videotaped procedures. One of these studies has now concluded, showing excellent validity (reported separately) and another is ongoing.

VNESS is a promising system for description of endometriosis severity and can potentially be useful for audit and research purposes by providing a reproducible system that allows benchmarking. It also can be an easy-to-use tool for communication between healthcare professionals and surgical documentation.

P127- Establishing a one-stop ambulatory hysteroscopy service using vaginoscopy at a large Acute Trust - outcomes, learning curve and patient acceptability

Richard Keedwell[1*], Jonathan Lord[1], Edward Keedwell[2]
[1]*Royal Cornwall Hospital Truro Cornwall UK;* [2]*University of Exeter Exeter UK*

Ambulatory hysteroscopy has been shown to be an efficient, cost-effective and acceptable method of gynaecological diagnostics and therapeutics. Barriers to the implementation of this service may include lack of training and skills, and concerns about patient acceptability. In this presentation we report establishing a successful ambulatory hysteroscopy service using a vaginoscopic approach in a large acute trust.

Data was collected over a 4 year period using a standardised database. Analysis was performed on over 1000 cases. Although the hysteroscopists had not previously had experience of outpatient hysteroscopy or vaginoscopy, success rates were satisfactory from the outset (87%) rising to 98%. Mean pain scores overall were almost identical for diagnostic and operative procedures with no learning curve effect. The pain scores for individual procedures are discussed and evaluated statistically. Also, exploration of the data using data mining techniques is performed.

Feedback taken from patients directly after their procedure showed 89% would elect to have an outpatient procedure again (3% not), with no difference in the first 6 months. A brief discussion of the economic situation surrounding the service will be included.

These data show that establishing a one-stop hysteroscopy service in a large acute trust can be achieved successfully even where clinicians have not previously had experience of vaginoscopy. We recommend the establishment of a similar service in other trusts, such that the economic and patient benefits may become more widespread.

ABSTRACTS

INDEX BY FIRST AUTHOR'S SURNAME	VIDEO POSTER NO
Ali O	FCVP22
Ali O	FCVP29
Ali O	FCVP31
Arambage K, Roman H, Pasquier G, Resch B, Huet E	FCVP28
Asfour V, Banerjee S	FCVP11
Baxter A, Oktaba M	FCVP16
Bean E	FCVP17
Bean E, Doufekas K, Salman G, Cutner A, Jurkovic D	FCVP25
Bharathan R, Gore R, Connell R, Kovoor E	FCVP26
Derpapas A, Khazali S	FCVP14
Derpapas A, Khazali S	FCVP15
Elsayed S, Szetho WZ, O'Sullivan R	FCVP18
Eskandar O, Diyaf A	FCVP6
Fayyad A, Onifade D, Pandeva I	FCVP19
Ghosh D, Aust T	FCVP30
Hardcastle R, Guyer C	FCVP8
Hutchinson L, Sircar S, McMurray D, Datsun K, Allam M	FCVP7
Ibrahim S, Sokolova I, Hawthorn R, Agur W	FCVP5
Ind T, Arshad I, Nobbenhuis M	FCVP4
Ind T, Heath O, Hookway T	FCVP3
Keedwell R, Sreekumar R, Byrne D	FCVP2
Kopeika J, Hamilton J, Kunde K	FCVP23
Kwasnicka LE, Penketh R, Datta S, Bruen E	FCVP20
Ma K, Hemers J, Morris R, Majumder K, Edi-Osagie E	FCVP21
Ma K, Majumder K, Clayton R	FCVP12
Minas V, Ahsraf K	FCVP24
Najdy M, Allam M, Elsapagh K	FCVP27
Nargund A, Griffiths A	FCVP13
Pandey S, Banerjee S	FCVP10
Pandey S, Khazali S	FCVP9
Sreekumar R, Byrne D	FCVP1

INDEX BY FIRST AUTHOR'S SURNAME	POSTER NO
Abdallah K	P98
Abery E, Delchar L	P89
Adishesh M, Kundodyiwa T	P68
Agrawal N, Al-Inizi S	P113
Ali O	P110
Ali O, Moukarram H	P96
Ali R, Mavrelos D, Saridogan E	P100
Ankers D, Coughlin L, Swaminathan A	P85
Arambage K, Visvanathan D	P9
Arshad I, Nath R, Austin C	P74
Asfour V, Fraser G, Banerjee S	P20
Banerjee S, Misra G	P71
Banerjee S, Misra G	P78
Bavananthan T, Lowden H, Arya P, Pathak M	P15
Bean E, Holland T, Cutner A, Saridogan E	P3
Beardmore-Gray A, Holland T, Vashisht A, Saridogan E, Cutner A	P76
Bharathan R, Kovoor E	P44
Bharathan R, McLaren J, Ind T	P59
Bharathan R, Teo L, Farag M, Barton D, Bulmer P	P41
Boland M, Broadbent M	P38
Bolton H, Nosib H, Majmudar T, Abdel-Rahmen H	P57
Borley J, Richardson R	P79
Brewer C, Wilson C, Oxby C	P87
Bullen N, Browning M, Coleman M, Frappell J	P2
Chandrakala Sathish, Matteo De Martino, Stanley Okolo	P92
Clarke J, Taghinejadi N, Craddock C, Holland T, Cohen R, Saridogan E, Cutner A	P111
Cooper N, Gbegbaje M, Ball E, Okaro E	P121
Cowan F, Shirol V, Burnham A	P90
Daniels J, Champaneria R, Shah L, Gupta J, Moss J, Birch J, Middleton L	P114
Datsun K, Elsapagh K, Allam M	P117
Davies M, Pandey S, Banerjee S, Khazali S	P35
Dickson M J	P5
Dickson M J	P37
Dilley J, Lo J	P23
Dodia R, Obura T	P109
Edirisinghe D, Yasmin E	P97
Eskandar O	P1
Eskandar O, Erfan I	P60
Fani Gkrozou, Eve Gaughan, Richard Pyper, Bronwyn Middleton, Natasha Waters	P46
Faraj R, Govinden G, Yeeles H	P26
Gaughan E, Gkrozou F, Middleton B, Pyper R, Waters N	P83
Gelson E, Bolton H, Pradhan A	P45
Gent D, Henderson D,Phillips K, Watson A, Ahmad G	P56
George S, Thomas A, Hale R	P64
George S, Dzyra J, Rai S, Pickersgill A, Llewinsky M	P82
Ghosh D, Rowlands D	P25
Gina Michel, Bronwyn Middleton	P99
Gkrozou F, Gaughan E, Middleton B, Pyper R, Waters N	P119
Gkrozou F, Gaughan E, Pyper R, Middleton B, Waters N	P50
Gnanachandran C, Woollett P, Kimani P, Mylvaganam K, Patel A, Izzat F	P12

TITLE

Combined laparascopic ovarian tissue cryopreservation and immature oocyte retrieval followed by in vitro maturation and vitrification for fertility preservation: Uk pilot study results

STUDY QUESTION

To study the feasibility of oocyte retrieval and in vitro maturation (IVM) in oncology patients undergoing ovarian tissue cryopreservation (OTCP) for fertility preservation

Summary Answer

The pilot study revealed that IVM of immature oocytes followed by vitrification could be successfully achieved in some oncology patients undergoing OTCP

What is known already

Previous studies, outside the UK, have already shown that the combination of ovarian tissue cryopreservation and immature oocyte retrieval followed by vitrification is a feasible option, irrespective of the phase of menstrual cycle or age of the patient. This is the first time this approach has been attempted in the Uk in both paediatric and adult patients to try to maximise fertility preservation.

Study design,size,duration

Prospective pilot study of 23 oncology patients (both paediatric and adult, age range 2-31) from 2013-2015. Laparascopic immature oocyte retrieval and removal of ovarian tissue for cryopreservation was scheduled at the time of insertion of the Hickman line, to avoid the patient undergoing multiple procedures.

Participants/materials, settings,method

All patients undergoing laparascopic ovarian tissue harvesting before cancer therapy were offered immature oocyte retrieval. Oocytes were retrieved by three methods: - in situ percutaneous video-assisted oocyte retrieval for those patients undergoing ovarian cortical strip resection (rather than oophorectomy), ex-situ puncturing of the excised ovary and removal of the fluid remnants post dissection and tissue processing of the ovarian tissue. Oocytes were then matured in vitro using standard IVM methodologies and any resultant mature oocytes vitrified.

Main results and role of chance

Of the 23 patients who participated in the pilot study, 15/23 (65%) were paediatric (age 2-17) and 8/23(35%) adults (age 22-31). Successful immature oocyte retrieval was achieved in 78% of the patients, 80% in the paediatric v 75% in the adult group, with the majority of the oocytes being isolated from the dissection remnants (61%). A total of 140 oocytes were collected, of which 57(41%) were degenerate and therefore non-viable. Of the remaining 83 oocytes, 40 reached metaphase (MII), giving a maturation rate of 48% following 24-48 hours in IVM culture. The remaining 43 (52%) arrested at germinal vesicle (GV) stage. All metaphase II oocytes were subsequently vitrified. Vitrification of mature oocytes was achieved in 60 %(9/15) of the paediatric

patients (range 1-5), the youngest of which was aged 2 (1 frozen), in comparison to 38 % (3/ 8) in the adult group.

Limitations, reasons for caution

None of the oocytes vitrified in this study have been thawed and therefore their viability is at present unknown. Also, due to restrictions in both the timings and collection methods of the oocytes the quality of the oocytes may be compromised as the main priority was OTCP.

Wider implications of the findings

This pilot study showed that immature oocytes can be successfully harvested /matured and vitrified from both antral follicles and excised tissue in both paediatric and adult patients undergoing OTCP. This is in accordance with other published studies .However, this study has also demonstrated freezing for the youngest patient to date.

Study funding/competing interest

Part NHS/charity funded patient participation.

No competing interests

Key Words

IVM, paediatric, oncology, cryopreservation,

Journal of Assisted Reproduction and Genetics, Vol. 21, No. 11, November 2004 (© 2004)

Piece of Mind

Should Ovarian Tissue Cryopreservation Be Recommended for Cancer Patients?[1]

Joseph G. Schenker[2,3] and Mohammad Fatum[2]

The emergence of ovarian tissue cryopreservation (OTCP) for fertility conservation has led to a new worldwide trend of ovarian tissue banking for reproductive cancer patients, scheduled to undergo chemotherapy or radiotherapy (1). Since the survival of young women and children who undergo such curative anti-cancer treatment is increasing, it is imperative for the consulting clinician to have an updated and accurate understanding of the proven benefits and the limitations of this new and hitherto evolving technique. OTCP was shown to be successful in several animal models during the last decades. In 1960, Parrott reported successful pregnancies in mice after implantation of frozen-thawed ovarian grafts (2). Other studies reproduced the similar results in mice (3) and in sheep (4). Despite these promising preliminary animal outcome, the efficiency of ovarian tissue autografting whether orthotopic (the autotransplantation to the ovarian pedicle) or heterotopic (the autotransplantation to a different site) has not yet been clearly demonstrated in humans (5,6).

Since the primordial and primary follicles can survive the freezing–thawing procedures, it was hoped that in vitro maturation would be an important source for harvesting mature oocytes (7,8). However, the in vitro maturation of both animal and human follicles was no less disappointing than autografting. There still are no reports of harvesting mature oocytes from in vitro culturing and maturation of primordial and primary follicles. Hence, there is increasing interest in improving the cryopreservation, autografting, and the in vitro culturing and maturation techniques, necessitating further intensive research to increase their efficiency and to test their safety (9). To our knowledge, no pregnancy or embryo transfer was reported in humans as a result of utilizing cryopreserved ovarian tissue.

From the aforementioned data, it seems that the use of ovarian tissue banking as a method to preserve fertility in cancer-treated-female patients is still in its early stages. We believe that at this stage of experience and outcome, OTCP should not be proposed to these patients. These relatively debilitated cancer patients are a priori in a suboptimal health state and hence, the risk of invasive surgical procedures for retrieving ovarian tissue is not justified.

Women should be informed of the current state of data concerning the success rates in order to prevent developing false expectations and further disappointments.

Furthermore, ovarian transplantation might be unsafe in some malignancies (e.g. acute leukemia) because of the risk of ovarian involvement and the possible receding of malignant cells through the implant (10,11). On the other hand, studies have shown that ovarian tissue harvested before high dose chemotherapy for lymphomas may not carry a risk of disease transmission by autotransplantation (12,13). However, even in these low risk cases, transmission of the malignant cells is difficult to exclude completely. Thus, if autografting is considered, testing for malignant cells in the tissue must be performed using adequate techniques. This subject needs further extensive research.

Animal models show that ovarian slices can convey short time function only. Assuming that these studies apply to human ovarian tissue, the potential of achieving pregnancy is limited. For this reason it is recommended to transplant the auto-graft only when immediate fertility is desired (11).

Studies on organ cryopreservation, ovarian freezing, and transplantation are in its first steps of research

[1] As of the date this paper was submitted, Donnez et al. (Lancet, Oct. 2004) reported a live birth after orthoptic transplantation of cryopreserved ovarian tissue.

[2] Department of Obstetrics and Gynecology, Hadassah, Hebrew University Hospital, Ein kerem, Jerusalem, Israel.

[3] To whom correspondence should be addressed at The Department of Obstetrics and Gynecology, Hadassah University Hospital, Ein Kerem, P.O. box 12000, Jerusalem 91120, Israel; e-mail: schenker@cc.huji.ac.il.

1058-0468/04/1100-0375/0

and thus it is not an applicable modality and cannot be recommended as a fertility-conserving measure in the near future. Since OTCP is yet to be yielding and in the absence of other more promising modalities, we believe that in order to preserve potential fertility in cancer patients, they should be advised to undergo one cycle of ovarian super-ovulation with subsequent IVF before chemotherapy or irradiation in order to obtain embryos for cryopreservation. This strategy can be suggested to stable couples, since fertilization can be performed with the partner's sperm.

These frozen zygotes have an acceptable rate of viability and so far are the only proven modality ensuring delayed pregnancy. To our experience one cycle of ovarian super-ovulation knowledge, is adequately safe and is equally acceptable by these patients and their consulting oncologists in most malignancies without any significant deleterious influence of the delay in chemotherapy/radiotherapy on patients' prognosis.

In single adolescent women, donor sperm can be used for oocyte fertilization. In accordance with their desires, both couples and single women will always have embryo transfer as an option. According to the present state, embryos can be cryopreserved for 5 years and in some countries according to legislation or regulation, even to 10 years, then women can decide whether they are interested in the sustained cryopreservation and transfer of these embryos.

With patients refusing to use donor sperm due to ethical or religious attitudes, oocyte harvesting and freezing might be offered despite present low fertilization and pregnancy rates. This modality is still in its early experimental stages and only few cases of pregnancies were achieved, however it is still more promising than OTCP in which no cases of pregnancies were reported.

Until large-scale human transplantation studies to test and improve the efficacy and the safety of OTCP procedure are undertaken, this procedure should not be recommended as a routine therapeutic approach. The current experience with no documented pregnancy in humans from OTCP, does not justify exposing these patients to the risks of anesthesia and invasive surgical procedure before the definitive anticancer treatment. This is of utmost importance in order to avoid creating false expectations and disappointments in these patients. Therefore, these women should be recommended to undergo one cycle of ovarian super-ovulation with subsequent IVF with partners sperm in coupled women and donor sperm in single women in order to achieve embryos for cryopreservation.

However, only in prepubertal girls in whom ovulation induction can not be performed and mature oocytes can not be achieved, OTCP is at present the only option. These patients and parents ought to be informed that this clinical approach is still in its early stages of experimentation and their chance to preserve fertility can not be guaranteed.

REFERENCES

1. Meirow D, Fasouliotis SJ, Nugent D, Schenker JG, Gosden RG, Rutherford AJ: A laparoscopic technique for obtaining ovarian cortical biopsy specimens for fertility conservation in patients with cancer. Fertil Steril 1999, May;71(5):948–951
2. Parrott DM: The fertility of mice with orthotopic ovarian grafts derived from frozen tissue. J Reprod Fertil 1960;1:230–241
3. Carroll J, Gosden RG: Transplantation of frozen-thawed mouse primordial follicles. Hum Reprod 1993;8:1163–1167
4. Gosden RG, Baird DT, Wade JC, Webb R: Restoration of fertility to oophorectomized sheep by ovarian auto-grafts stored at −196 C. Hum Reprod 1994;9:597–603
5. Radford JA, Lieberman BA, Brison DR, Smith ARB, Russel SA, Watson AJ, Clayton JA, Harris M, Gosden RG, Shalet SM: Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 2001;357:1172–1175
6. Oktay K, Karlikaya G: Ovarian function after transplantation of frozen, banked autologous ovarian tissue [letter]. N Engl J Med 2000;342:1919
7. Hovatta O, Silye R, Krausz T, Abir R, Margara R, Trew G, Lass A, Winston RM: Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. Hum Reprod 1996;11:1268–1272
8. Oktay K, Nugent D, Newton H, Salha O, Chatterjee P, Gosden RG: Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue. Fertil Steril 1997;67:481–486
9. Picton HM, Gosden RG: In vitro growth of human primordial follicles from frozen-banked ovarian tissue. Mol Cell Endocrinol 2000;166:27–35
10. Shaw JM, Bowles J, Koopman P, Wood EC, Trounson AO: Fresh and cryopreserved ovarian tissue samples from donors with lymphoma transmit the cancer to graft recipients. Hum Reprod 1996;11:1668–1673
11. Oktay K: Ovarian tissue cryopreservation and transplantation: Preliminary findings and implications for cancer patients. Hum Reprod Update 2001;7:526–534
12. Kim SS, Radford J, Harris M, Varley J, Rutherford AJ, Lieberman B, Shalet S, Gosden R: Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. Hum Reprod 2001;10:2056–2060
13. Meirow D, Ben Yehuda D, Prus D, Poliack A, Schenker JG, Rachmilewitz EA, Lewin A: Ovarian tissue banking in patients with Hodgkin's disease: Is it safe? Fertil Steril 1998, Jun;69(6):996–998

Introducing a centralised tertiary programme for ovarian tissue cryopreservation in female cancer patients. Biomedical, ethical, regulatory and funding challenges.

Muhammad Fatum, MD, Christian Becker, MD, Enda McVeigh, MD, Jill Davis, MSc, Kokila Lukhor, MD, Karen Turner, PhD, Stephen Kennedy, MD, Tim Child, MD, Sheila Lane, MD,

Introduction

Introducing a nationwide ovarian tissue cryopreservation programme (OTCP) for female cancer patients, an innovative technology based upon largely experimental technology developed elsewhere in the world, is a challenging task with a number of scientific, ethical, regulatory and funding implications[1,2]. We report these challenges when introducing a new scheme for ovarian tissue cryopreservation, achieved through collaboration between Oxford University, Oxford University Hospitals NHS Foundation Trust and the private sector.

Across the world, more than 300,000 children under the age of 19 are diagnosed with cancer each year[3]. Overall survival for children and young adults following cancer treatment in high-income countries now exceeds 80%[4]. However, the treatments required to achieve these very encouraging survival figures are associated with significant morbidity: for example 1 in 10 women who survive cancer face premature ovarian insufficiency (POI) and sterility at a very early age before they have had children[5,6]. With the increasing awareness of patients, guardians and clinicians on the reproductive and endocrine disrupting effects of chemotherapies, there is an increasing need and demand to thoroughly discuss these issues with patients and offer them satisfactory fertility preserving treatments prior to commencing the chemotherapy/radiotherapy[7].

Currently, there are several well established fertility preservation options in females which are highly dependent on the physiologic age of the female[8,9]. Firstly, post-menarchial girls or post-pubertal adults can be offered in vitro fertilization (IVF) for either embryo or oocyte cryopreservation. The main advantage of this fertility preservation modality is that it is an established technology and is in routine clinical use in IVF units throughout the world on a daily basis. The major drawback of this option is the need for almost two weeks of hormonal stimulation followed by egg collection with their inherent side effects and complications. In addition, it is a relatively expensive treatment and not routinely NHS funded which adds to the difficulties these patients endure. Furthermore, only a limited numbers of embryos/oocytes can be stored from a single or couple of stimulation cycles. Secondly, a less common modality and less effective option is in vitro maturation (IVM) followed by embryo or oocyte vitrification[10]. The main advantage of this technique is that patients do not need ovarian stimulation and its related adverse effects and complications, especially ovarian hyperstimulation syndrome OHSS. Thirdly, ovarian suppression by monthly injections of GnRH analogues is occasionally prescribed prior to commencement of chemotherapy. Its efficiency in fertility preservation is still controversial and its use should be offered only in well-designed research-based experimental protocols[11]. Fourthly, fixation of ovaries (Oophoropexy) outside the pelvic radiotherapy field may be appropriate in a small subgroup of patients who need to undergo pelvic brachytherapy or radiotherapy where it is possible to

mobilise the ovary and move it without compromise to ovarian blood supply. Success of oophoropexy is very variable[12].

Ovarian tissue cryopreservation is an emerging fertility preservation technology that involves the procurement of ovarian tissue either as ovarian cortical biopsies or unilateral oophorectomy, followed by processing of this tissue into small slices and cryogenic freezing in a controlled freezing programme. Laparoscopy is usually performed to procure ovarian tissue either by unilateral oophorectomy or ovarian biopsies. Following the patient's recovery from cancer treatment and when they are ready to start a family, if they have developed POI (Premature Ovarian Insufficiency), the ovarian tissue can be thawed out and autotransplanted to the patient. Once blood supply is reestablished and tissue proves viable, folliculogenesis is resumed with restoration of natural fertility and endocrine ovarian activity. The tissue is commonly transplanted in or near its natural site, such as the ovarian hilum, ovarian medulla, broad ligament or the pelvic sidewall (orthotopic transplantation). This has been shown to offer the potential for spontaneous pregnancy as eggs are left in their physiologic environment, in the proximity of the fallopian tubes, thus obviating the need for IVF treatment[2,11,13-14]. Alternatively, heterotopic transplantation which involves auto-transplantation of ovarian tissue into non-native ectopic locations such as the arm or abdomen has also been attempted[15,16]. Heterotopic transplantation has certain advantages such as an easier approach for follicular monitoring and egg retrieval for IVF, but, this approach is less commonly utilized. Orthotopic

transplantation has been reported to result in more effective revascularization and less follicle loss and consequently is believed to be more effective[17,18]. Orthotopic autotransplantation is performed either by laparotomy or laparoscopy. Several ovarian cortical slices are thawed out and then either sutured to the remaining ovary or left unsutured placed into a peritoneal window. To date, more than 100 pregnancies have been reported after autotransplantation of ovarian cortical tissue slices mainly after orthotopic approach[17,18]**.** Published reports indicate a time interval of 3.5 to 6.5 months after the autografting before endocrine function is restored (ie decreasing FSH levels) or folliculogenesis are detected. Ovarian endocrine activity was observed in over 95% of patients after autografting[19]. The post-transplantation pregnancies reported thus far included natural conceptions, ovulation induction cycles and IVF cycles**.** Ovarian tissue grafts have been reported to maintain endocrine function for up to 10 years[20] and is mainly dependent on the ovarian reserve before the harvesting, the absence of chemotherapy before cryopreservation and the degree of ischemia after re-implantation[21]. These promising results strongly suggest that Ovarian Tissue Cryopreservation (OTCP) is a viable fertility preservation option that should be part of any oncofertility programme.

OTCP is the only available fertility preservation approach for premenarchial/prepubertal girls and for adults who need to promptly start their chemo/radiotherapy and who cannot undergo an IVF cycle and delay their cancer treatment for 3-4 weeks. OTCP can be performed immediately without any delay, as no hormonal stimulation is needed, and in children and young adults potentially thousands of follicles exist

in each small cortical slice, thus conferring a quantitative advantage. In this report we present our collaborative results and discuss the diverse challenges encountered during the establishment of the first OTCP service in England including the medical, scientific, legal/regulatory and financial ramifications.

Materials and Methods

Patient enrolment:

Following approval from the Human Tissue Authority (HTA), the Human Fertilization and Embryology Authority (HFEA) and the new Technologies Advisory Group at Oxford University Hospitals NHS Foundation Trust (OUHFT), we commenced recruitment of suitable patients. Patients were largely referred by their treating oncologists and acceptance for ovarian tissue cryopreservation, was accessed on clinical information and by reference to the service eligibility criteria as listed in the PPD (Preparation Process Dossier) which was submitted to the HTA. Once the indication for OTCP was established, informed consent was obtained. For minors under the age of 18 years, parents or guardians were required to sign a written consent form. Patients are routinely screened for mandatory viral serologic screening tests including HTLV1/2, HIV1/2, HBV and HCV which need to be taken at procurement.

Indications:

Our ovarian tissue cryopreservation programme eligibility criteria were:

1. Female patients aged between 12 months – 40 years who are unable to have egg/embryo storage and who are at significant/high risk of POI. Later, the age range was redefined to 0-35 years, as no live births have been reported in tissue collected from women over 35 years.
2. Prognosis – curative treatment intent and OTCP procedure possible without an unacceptable delay to definitive treatment i.e. within 7 days of referral.
3. Patients unable to store eggs/embryos
4. Patient fit enough to undergo general anaesthetic and surgery.

The lead consultant for OTCP reviewed all referrals. Complex referrals or cases that fell outside the referral criteria are discussed at the monthly OTCP (MDT) board meeting which was held on a regular basis or virtually when an urgent response was needed. The MDT was comprised of a paediatric oncology consultant, paediatric and reproductive surgeons, an IVF specialist, consultant ethicist when needed, the Person Responsible for the HTA and Designated Individual for the HFEA license for the service and University researchers. The MDT , oversees the scientific, clinical, financial and research management of the programme.

The referred patients' chemotherapeutic protocols and irradiation programmes were carefully studied in order to estimate the gonadotoxicity of the planned treatment. This risk

quantification was based on the best evidence as reflected in the most up to date pertinent literature. In addition, a subgroup of females who had non-malignant conditions that put them at risk of POI, i.e. a family history of POI or autoimmune diseases requiring high dose cytotoxic treatment or patients requiring Bone Marrow Transplantation for benign haematological diseases such as thalassemia major, were added to the indications as the programme developed. This board was set up as part of the detailed HTA license extension and the Preparation Process Dossier (PPD) submission.

Following each patient referral, a mandatory thorough pre-treatment consultation by at least one OTCP consultant was performed.

Tissue Collection, processing and storage:

Ovarian tissue harvesting:

Ovarian cortical tissue procurement was done after the consenting process. In preference, it was performed as a laparoscopic procedure under general anaesthesia. If open surgery was planned for the patient, ovarian biopsies or oophorectomy were performed at the time of the planned surgery. In order to save the patient to needlessly undergo an additional general anaesthesic, the ovarian harvesting procedure was combined where possible with other elective procedures: e.g. the insertion of Hickman catheter prior to starting chemotherapy. Unilateral oophorectomy was commonly performed in order to ensure an adequate amount of tissue was

taken and to reduce the risk of post-operative bleeding. Alternatively, ovarian biopsies were taken with laparoscopic scissors. The surgeon decided whether to perform a unilateral oophorectomy or less commonly resect several 15mm long and 5mm wide cortical strips from each ovary. Any resultant bleeding was best controlled with small, precise bipolar diathermy. As excessive use of diathermy might cause thermal damage to the ovarian tissue, it was important to minimize the use of diathermy in the proximity of the ovary. In addition, the surgeon must gently handle the tissue and refrain from any untoward trauma to the ovarian tissue and to promptly extract the tissue and aseptically transfer directly into sterile cooled transport fluid in a labelled container held by the Oxford Cell and Tissue Bank's (OCTB) technician attending.

Tissue Processing and storage:

After procurement, packaging and labeling the ovary or biopsies were transported from the operating theatre to the OCTB licensed cleanroom facility by the OCTB attending technician in cooled transport media. For tertiary sites the tissue was transferred within 6 hours to the OCTB. The laboratory protocols for the processing and storage of ovarian tissue are essentially as has been described elsewhere in detail[22,13]. In keeping with the requirements of the HTA Licence the tissue was processed in the grade A processing suite according to the protocol

detailed in the PPD[22]. A slow freeze protocol was adopted to satisfy the HTA regulations and because most live births have been reported in programmes using this technique[13].

Supraregional referrals and third party contracts:

In the first phase of the programme referrals were from patients within the Oxford and Thames Valley cancer networks. As the programme matured, increasing number of cases were referred from outside the local catchment area. Initially all cases had surgery in Oxford but more latterly we developed a 'hub and spoke' model whereby patients could have surgery at their local hospital with samples being couriered to Oxford for processing and storage. This arrangement required HTA approval and the development of third party agreements with each 'spoke. A "Third Party Agreement" (TPA) confirming roles and responsibilities for each party involved is mandatory to ensure that the statutory and regulatory requirements for procurement of ovarian tissue are met, and was put in place.

Database development

A digital database was developed onto which all patients were registered upon acceptance of referral. It included epidemiological, clinical, surgical information obtained from referring consultants, medical and surgical records. The tissue procurement, processing

and testing details were also recorded. This also allowed capturing and recording of all serious adverse events (SAE) that must be reported to HTA within 24 hours.

Results:

Our goal from the current report is to prove the feasibility of introducing OTCP as a fertility preservation modality in a well-defined range of indications. Prior to the launch of the Oxford Service, ovarian tissue cryopreservation was only available in the UK as part of a research programme running in Scotland. However, with the increasing evidence concerning its efficacy, including ever growing numbers of livebirths worldwide, we believe this technology should be made available as an experimental medicine, clinical service for patients at high risk of infertility who were unable to store eggs and embryos. In the present paper, we report our experience in service development and discuss the different challenges met.

Referrals:

During the first three years of patients enrolment to the programme, 100 patients were referred for ovarian tissue cryopreservation. At the time of processing the data from the first

100 patients , five patients were not offered tissue collection as they did not fulfill eligibility criteria, 5 patients or their families declined tissue collection after the initial consultation and 4 patients were still awaiting surgery. The results from the 86 patients undergoing tissue collection in the first 3 years of the programme are included in this paper.

The majority of patients (n=82) were operated in OUHFT either by a gynaecologic surgeon at the Women's Centre at the John Radcliffe Hospital (n=24) or by a Paediatric surgeon, Oxford Children Hospital (n=57), with one patient operated by gynae-oncologist at Churchill Hospital in Oxford. Four patients, were operated in other supraregional hospitals and the tissue was transferred to OCTB within 6 hours, with three of them operated by gynae-oncologist and one operated by a general surgeon.

The majority of the operations were done laparoscopically (n=81) and the rest (n=5) where done in an open approach whilst patients were having open procedures to treat their primary disease.

Ages:

The majority of the recruited patients were paediatric and adolescents patients (up to but not including 19th birthday) (n= 60, 69.8%) and 26 patients (n=26, 30.2%) were adult patients. Fig.1 shows the age distribution of patients undergoing the ovarian tissue

cryopreservation.

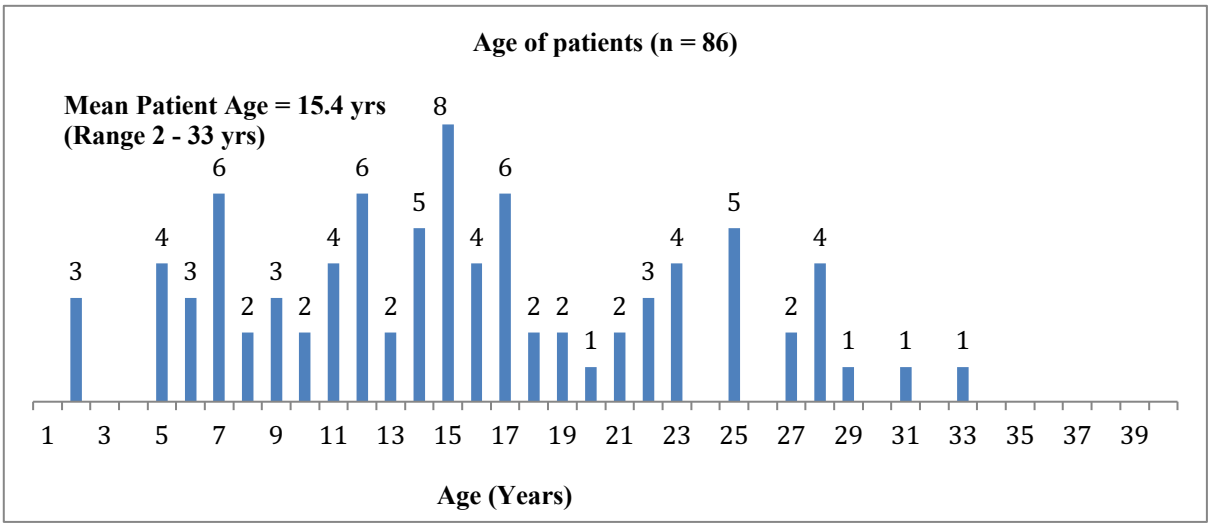


Fig.1 The age distribution of patients undergoing OTCP.

Indications:

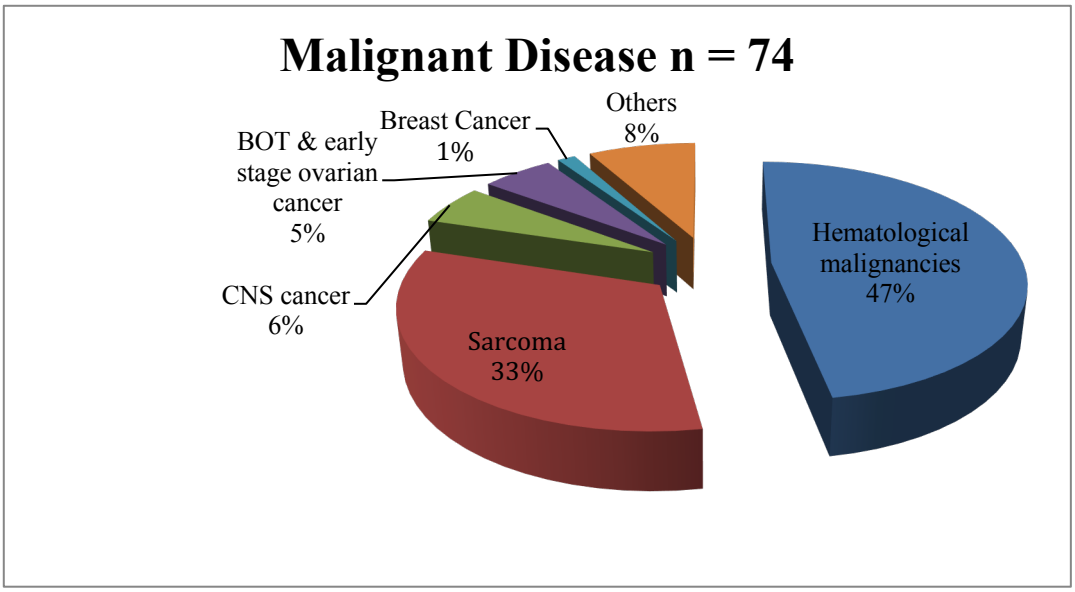
The majority of the patients who had their ovarian tissue cryopreserved were oncological patients (n=74, 86%) and the rest (n=12, 12%) underwent the procedure as they were having bone marrow transplant and therefore high dose conditioning treatment to cure their underlying benign disease. All patients were at high risk of POI due to chemotherapy or radiotherapy they were planned to have for the cancer treatment or before bone marrow transplantation (BMT).

Malignant diseases:

Of the first 100 referred patients, the vast majority were patients diagnosed with malignancies, while the rest were referred due to benign diagnosis before the commencement of cytotoxic treatment.

In the current series, 74 patients underwent ovarian tissue cryopreservation for malignant disease that posed the at high risk of POI, (see Fig. 2). Haematologic malignancies constituted the main indication (47.3%, n=35) and included: relapsed/resistant Leukemias , high risk Hodgkin's Lymphomas and non-Hodgkin Lymphoma. The second main indication was Sarcomas (32.4%, n=24). Other indications included CNS tumors (5.4%, n=4), Borderline ovarian tumor (BOT)and early stage ovarian cancer (5.4%, n=4), Breast carcinoma (n=1, 1.4%), Colorectal carcinoma (1.4%, n=1), Peritoneal papillary serous adenocarcinoma (1.4%, n=1), Nasopharengeal carcinoma (1.4%, n=1), Wilm's tumor (1.4%, n=1), mixed ovarian teratoma (1.4%, n=1), Synovial carcinoma (1%, n=1).

Figure 2. Pie chart showing the different underlying diagnoses of participant patients and their percentage.



Benign diseases:

Ovarian tissue cryopreservation prior to BMT for benign disease was undertaken in 12 patients and constituted 14% of all patients. The diagnostic categories were sickle cell anemia before bone marrow transplantation (41.8%, n=5). Other indications included: Beta Thalassemia major prior to bone marrow transplantation (25%, n=3), Autosomal recessive chronic granulomatous disease prior to bone marrow transplantation (8.3%, n=1), NMDA receptor encephalitis (8.3%, n=1), Blepharophimosis, ptosis, and epicanthus inversus syndrome (BPEI) (8.3%, n=1)

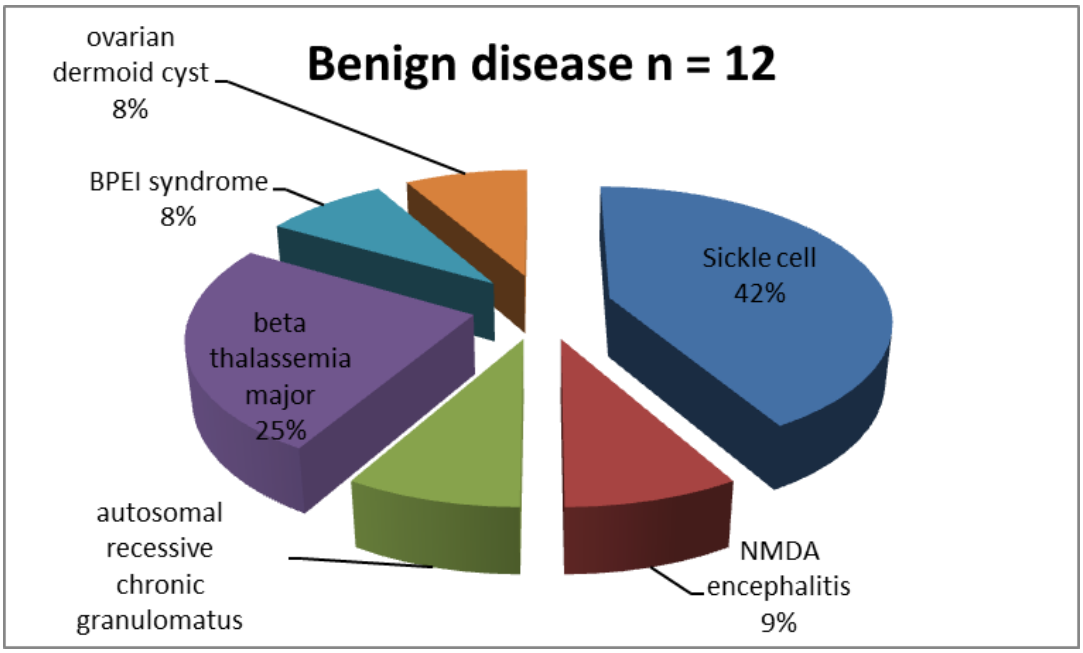


Figure 3. Patients who underwent ovarian cryopreservation for benign indications (percentage of all benign diagnoses).

Surgical procedure:

Laparoscopy was the main and preferred operative approach applied to harvest ovarian tissue (n=81) whilst the rest (n=5) had open surgery performed for the planned definitive surgery for the cancer treatment. All procedures were uneventful. In one patient a surgical wound infection was observed, successfully treated with intravenous antibiotics.

Discussion:

We report on the establishment of the first children and young adult fertility preservation programme in England and the outcomes of the first three years since its advent and development. Up to the launch of the service at Oxford University Hospitals NHS Foundation Trust and offering this as a clinical application, OTCP was only available as part of a research programme in Edinburgh[23]**.** Following the worldwide reporting of successful outcomes of ovarian tissue autografting[24], we designed a stepwise scheme that included initially a regional and then a countrywide service that could be applied within a clinical setting, in addition to starting different research programmes to advance the technology and address the challenges. This article presents the outcomes achieved, the challenges met and the future prospects.

Indications

We report our series of 86 patients undergoing OTCP who fulfilled the strict inclusion criteria. Initially the majority of patients were regional referrals. As the programme became more established, we developed a countrywide framework to respond to the growing interest and need from extra-regional trusts.

The indication for ovarian tissue cryopreservation was restricted to patients at high risk for POI. Initially the hematological indications constituted the main group (47.3%, n=35),

whereby relapsed and resistant Leukemias were the most common, followed by high risk Hodgkin's Lymphomas and non-Hodgkin Lymphomas. The second main indication was Sarcomas (32.4%, n=24). These indications and the other less common indications are presented in the pie chart in fig 2, and are a direct reflection of the common cancers in the paediatric, adolescent and young adults. This population of patients enrolled in the current report may change in future as patients awareness of the service increases and as oncologists and other care providers allocate more time and resources to fertility preservation for young patients. Other series showed a similar spectrum of indications with haematological indications being one of the main indications[25]. Similarly sarcomas were also common as an indication of ovarian tissue preservation[26]. Sarcoma patients are at high risk of early infertility due to the gonadatoxic chemotherapy they receive , as with any children and young adult patients even if they are old enough to store eggs they cannot delay start of treatment for several weeks.

Of note, only one of our patients had breast cancer, in contrast to several other studies who reported varying numbers of breast cancer patients undergoing ovarian tissue cryopreservation[26]. This could be partially explained by different fertility preservation approaches in different countries for patients with breast cancer. Concern on the safety of ovarian stimulation in the context of IVF treatment for fertility preservation, especially in the presence of hormone receptor positive breast tumors remains. Consequently, while some fertility preservation programmes prefer OTCP for such patients, others, including our fertility preservation programme in Oxford, believe that there is insufficient evidence to suggest

prognosis-aggravation of a single IVF cycle for fertility preservation. As breast carcinoma is primarily an adulthood tumor, these patients are offered an IVF as their first best option.

As the programme progressed, more patients with benign indications were offered OTCP including benign haematological conditions prior to BMT and autoimmune conditions that require high doses of gonadotoxic chemotherapies. We anticipate this section of indications to increase in the future. One patient with BPES syndrome, a genetic condition associated with high risk of POI, was offered to undergo the procedure in an anticipation of looming future gonadal failure. This genuinely opens up a new horizon of fertility preservation for a wide range of genetic and chromosomal conditions known to predispose to early ovarian failure.

The success in setting up OTCP service in Oxford University Hospitals Trust, despite the lack of central governmental funding, sets a fascinating example of the ability of the medical community backed by charities and the private sector to initiate a change in health care. This is of critical importance especially as the main population is the paediatric and adolescent population, who are essentially dependent on their guardians, families and professional health carers. With the advancement of the programme locally, the service was opened to referrals from all over the UK, as will be discussed in more details below.

The emergence of this ever evolving fertility preservation technology, has necessarily met medical, financial and regulatory challenges that needed to be settled in order to run the programme.

Establishing a tissue bank:

One of the first challenges was to find a facility to bank ovarian tissue fulfilling for all licensable activity's regulations associated with human tissue cryopreservation. The HTA is the competent authority in the UK to monitor compliance of tissue banks with the Quality and Safety (Q and S) regulations transposing the European Union Tissue and Cells Directives (EUTCD's). A clean room, filtered and pressurized with high-efficiency particulate air (HEPA), is required, which must be continuously monitored to meeting environmental requirements as set out in Q & S regulations, and detailed in the Rules and guidance for pharmaceutical manufacturers and distributors. One option was to house the bank at the Oxford Fertility Unit but environmental monitoring requirements for processing of gametes in the UK do not stipulate use of cleanroom facility. IVF units therefore do not meet requirements set out in Q&S regulations. The second option was to request an HTA licensed tissue bank processing tissue for human transplantation within OUHFT to complete all required processes in order to submit an application to extend their licence to include ovarian tissue banking. We were fortunate that the Oxford Heart Valve Bank (OVHB) was already

operating within OUHFT with experienced scientific and technical staff and they had all the necessary equipment, clean rooms and storage facilities and agreed to apply to take on this additional tissue cryopreservation service. Later on, the tissue bank also submitted an application to further extend the OVHB licence to include immature testicular tissue cryopreservation.

HTA and HFEA

Understanding the current legal and regulatory status of ovarian tissue banking was of most importance at the early stages of service building. The regulatory complexity of OTCP stems from the fact that it consists of tissue preservation with the ultimate purpose to create gametes in the future. Henceforth, regulation of OTCP fell within the scope of both authorities, HTA and HFEA and tissue banks preserving ovarian tissue initially required licenses by both authorities. However, HFEA and HTA later published a joint statement confirming HTA license was sufficient for preserving ovarian tissue[27]. A tissue bank must first submit a dossier to the HTA before it can apply for a licence to store a new tissue type. A review of all published methods for ovarian tissue preservation was thoroughly done, together with direct collaboration with successful existing ovarian tissue programmes[13,23]. The Standard operating policies and procedures for tissue processing and cryopreservation were validated using porcine ovaries. A slow freeze protocol was adopted for freezing, as most programmes reporting live births have

used this technique. Post-thaw tissue morphology demonstrated preservation of ovarian follicles. OCTB also repeated post thaw assessment following cryopreservation for the first three human ovarian tissue samples. After meticulous validation of our protocols, demonstration of full compliance with UK requirements for ovarian tissue licensable activities, compliance with the requirements for gametes, and on-site inspection we were awarded an HFEA Licence in July 2013, followed by the initial conditional authorization to extend HTA licence for OTCP.

TAG approval for experimental medicine procedure:

Approval also had to be sought from the OUHFT Technologies Advisory Group (TAG), a committee responsible for conducting an independent appraisal of all proposed new technologies or procedures introduced into routine clinical practice or within an experimental medicine programme with the exception of drugs, which are addressed in a separate forum. In the context of the TAG committee, the term experimental medicine refers to any new technology/procedure be introduced to *'demonstrate proof-of-concept evidence for the validity and importance of new discoveries or treatments'*. TAG, which reports to the OUHFT Clinical Governance Committee, evaluates proposals in terms of patient safety, clinical effectiveness, staff training and competency, service improvement and the impact on other clinical areas. It does not consider the cost of new technologies/procedures, nor how they are funded.

Service Funding:

Despite 2007 NICE guidelines indicating the need of developing ovarian tissue cryopreservation (OTCP) for paediatric and premenarcheal females, albeit in a research based framework, this recommendation was not adopted by the NHS and no public funding was available to start the service[28]. The fact that at the time of launching the service, OTCP was not included in the NICE Fertility guidelines , was a major hurdle as Clinical Commissioning Groups had no requirement to fund the service and money was not available to fund set up. Supported by the increasing evidence of the efficacy of the technology, we decided to resort to charities to fund the introduction of the OTCP service in Oxford University Hospitals, where all needed biomedical disciplines coexisted, and started a well orchestrated multidisciplinary collaboration. The presence of an existing tissue bank facility with equipment and staff able to take on this extension of service was instrumental to reduce the costs and enable its launch. The current report is the culmination of a comprehensive collaboration between the University of Oxford, Nuffield Department of Obstetrics and Gynaecology, the Oxford University Hospitals NHS Foundation Trust, the Oxford Fertility Unit, the Oxford Cell and Tissue Biobank; the Department of bioprocessing and tissue bioengineering, Oxford University; the Department of Pathology, the Paediatric surgery and haemato-oncology departments at Oxford Children Hospital. As the programme expanded to patients from outside Oxford , those external referrals were done in a third party scheme at their local hospitals.

Patients pathways and criteria:

We decided to recruit patients with high (>80%) risk for POI undertaking chemotherapeutic protocols and irradiation regimens, based on the most uptodate pertinent literature. The inclusion criteria for oncologic patients are summarized in the methods section. As the programme develops and clinical data become available, we believe that ovarian tissue cryopreservation will be increasingly offered to patients with non-malignant diseases such as females at risk of POI: i.e. Turner syndrome, family history of premature ovarian insufficiency, benign autoimmune diseases requiring gonadotoxic chemotherapy or patients needing bone marrow transplantation for benign haematological diseases such as sickle cell anemia and thalassemia major.

Third party agreements:

In order to accommodate supraregional referrals we offered two options. The first option is to arrange for the patients to travel to OUH for their tissue procurement. The consent interview for tissue storage is then performed by the OUH OTCP lead consultants, the surgery by OUH trained surgeons and the tissue collected and transported and processed by the OCTB technical staff. The alternative service that we offer is to extend the OCTB HTA license to enable procurement at the patients local hospital. This involves OCTB working closely with HTA to address the technical challenges and to secure collaboration with the hospitals

operating theatres and with surgeons who will procure the tissue. Consent is taken by the OUH OTCP consultant, one of the OCTB technical staff attends the procurement, packages and transports the tissue back to OUH for processing. This also involved basic training of the procurement surgeons. The tissue processing is done at OCTB within 6 hours of procurement, although there is evidence from other international programmes that tissue may be successfully cryopreserved up to 24 hours afterwards . With these schemes, we are able to provide a service for patient referrals from all areas of England . These supraregional referrals are assisted either by the charitable funds which cover the processing and storage of the tissue or are privately funded as a last resort. Following the relative success of applying this technique at OUHFT, together with the heightened awareness of patients, their families, oncologists and health care providers and trusts, we hope to be able to secure funds to continue this service.

Centralizing service and data

As the programme aims at addressing and providing for the clinical need of OTCP services for oncological patients across England a website has been launched to aid patients, guardians and professionals in their pursuit to access our ovarian tissue preservation programme. Up to date information concerning the fertility preservation programme is provided for patients, together with clear referral system for healthcare professionals. With the increasing rate of referrals and procurement procedures done, it will probably be inevitably

important to produce a centralized database of all ovarian tissue preservation procedures and autografting done throughout the UK.

In summary, in the current report we have shown the feasibility of building up a countrywide scheme throughout England, Wales and Northern Ireland for ovarian tissue cryopreservation for cancer patients and other non-cancer patients who are at high POI risk. The introduction of this innovative fertility preservation technology, presented a wide range of scientific, medical, regulatory and financial challenges. Our scheme comprises of either direct referrals to OUHFT or by third party agreements with other regional hospitals, builds the foundations for offering this technology to all patients requiring the service from all over England, Wales and Northern Ireland. Future translational research projects will have pivotal importance to continue the development of this technique and answer the hitherto unsolved challenges. With the successful establishment and the growth of the clinical OTCP programme in Oxford, we hope to envisage and implement complementary basic and translational research projects to continue the development of this innovative technique and to meet the various challenges it brings up.

Strengths and Limitations of this study

- We have demonstrated the feasibility of building up an ovarian tissue bank for cancer patients.
- Our suggested model of either extra regional referral of patients to a tertiary center or by third party contracts with regional hospitals, offers flexibility to patients and health care providers.
- Our 'hub and spoke' model whereby patients could have surgery at their local hospital with samples being couriered to Oxford for processing and storage, can offer ovarian tissue cryopreservation to patients from all over the country.
- The study pertains to the early years of set up and indications and scope of work is dynamically changing, as patients and health care professionals awareness of the service increases and more time and resources are allocated for fertility preservation for young patients.
- Ovarian tissue cryopreservation is a novel technology and the use of frozen tissue is still in its early days and nationwide Consensus on indications and funding is yet to be agreed.

Abstract

Background: In the recent years, cryopreservation of ovarian tissue has emerged as a promising technique for fertility preservation in premenarchal children and adolescents, as well as an alternative in adult women prior to starting gonadotoxic chemo/radiotherapy. The prognosis and the survival rates of cancer patients have improved tremendously as a result of recent advances in cancer treatment - particularly in childhood cancers - and therefore attention is now being directed towards long-term gonadotoxic side effects of chemotherapy or radiotherapy. In the current report we aim at exploring the feasibility of establishing ovarian tissue banking in a tertiary hospital at John Radcliffe Hospital, Oxford University Hospitals trust.

Study population: Ovarian tissue cryopreservation has been offered to premenarchal, adolescent and adult females with cancer or benign conditions who were scheduled to get a high risk gonadotoxic treatment. The study was performed between August 2013 and July 2016.

Methods: In the first phase of the programme referrals were from patients within the Oxford and Thames Valley cancer networks. As the programme matured increasing number of cases were referred from outside the local catchment area. Initially all cases had surgery in Oxford but more latterly we developed a 'hub and spoke' model whereby patients could have surgery at their local hospital with samples being couriered to Oxford for processing and storage. In this study we present our scheme, methods, results and challenges of establishment of a new ovarian tissue bank including medical, scientific and financial ramifications.

Findings: We report the series of 86 patients undergoing ovarian tissue preservation treatment who fulfilled the strict inclusion criteria. The majority of patients were initially

regional referrals coming within the Thames Valley Trusts, then as the programme became more established, we developed a centralised referral framework to answer growing interest and need from extra-regional trusts. Seventy four patients underwent ovarian tissue cryopreservation for malignant disease. Haematologic malignancies constituted the main indication (47.3%, n=35) and included: Leukemias , Hodgkin's Lymphomas and non-Hodgkin Lymphoma. The second main indication was Sarcomas (32.4%, n=24). Other indications included CNS tumors (5.4%, n=4), Borderline ovarian tumor and early stage ovarian cancer (5.4%, n=4), Breast carcinoma (1.4%, n=1), Colorectal carcinoma (1.4%, n=1), Peritoneal papillary serous adenocarcinoma (1.4%, n=1), Nasopharengeal carcinoma (1.4%, n=1), Wilm's tumor (1.4%, n=1), mixed ovarian teratoma (1.4%, n=1), Synovial carcinoma (1.4%, n=1). Ovarian tissue cryopreservation due to benign diseases was done in 12 patients and constituted 14% of all patients. The main indication was sickle cell anemia patients before bone marrow transplantation prior (41.6%, n=5). Other indications included: Beta Thalassemia major prior to bone marrow transplantation (25%, n=3), autosomal recessive chronic granulomatous disease (8.3%, n=1), NMDA receptor encephalitis (8.3%, n=1), Blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) (8.3%, n=1).

Interpretation: In the current report we have shown the feasibility of building up an ovarian tissue cryopreservation bank for cancer patients. Our suggested scheme of either extra regional referrals to our ovarian tissue bank at John Radcliffe Hospital or by third party contracts with regional hospitals, build the foundations for offering this technology to all patients requiring the ovarian tissue preservation from all over the UK.

References:

1. Donnez J, Dolmans MM, Demylle D, et al. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 364: 1405-10.

2. Donnez J, Jadoul P, Squifflet J, et al. Ovarian tissue cryopreservation and transplantation in cancer patients. Best practice & research clinical obstretrics & Gynaecology 2010; 24: 87-100.

3. Steliarova-Foucher E, Colombet M, Ries LAG, Moreno F, Dolya A, Bray F, Hesseling P, Shin HY, Stiller CA; IICC-3 contributors. International incidence of childhood cancer, 2001-10: a population-based registry study. Lancet Oncol 2017; 18: 719-731.

4. Winther JF1, Kenborg L, Byrne J, et al. Childhood cancer survivor cohorts in Europe. Acta Oncol 2015; 54: 655-68.

5. Green DM1, Sklar CA, Boice JD Jr, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the Childhood Cancer Survivor Study. J Clin Oncol 2009; 27: 2374-81.

6. van Dorp W, Haupt R, Anderson RA, et al. Reproductive Function and Outcomes in Female Survivors of Childhood, Adolescent, and Young Adult Cancer: A Review. J Clin Oncol 2018; 36: 2169-2180.

7. National Institute for Health and Care Excellence (NICE, 2014), Cancer services for children and young people, Quality standard. nice.org.uk/guidance/qs55

8. Anderson RA, Mitchell RT, Kelsey TW, et al. Cancer treatment and gonadal function: experimental and established strategies for fertility preservation in children and young adults. Lancet Diabetes Endocrinol 2015; 3: 556-67

9. Sonmezer M & Oktay K. Fertility preservation in female patients. Hum reprod update 2004; 10: 251-266.

10. Creux H, Monnier P, Son WY, et al. Immature oocyte retrieval and in vitro oocyte maturation at different phases of the menstrual cycle in women with cancer who require urgent gonadotoxic treatment. Fertil Steril 2017; 107: 198-204.

11. Munhoz RR, Pereira AA, Sasse AD, et al. Gonadotropin-Releasing Hormone Agonists for Ovarian Function Preservation in Premenopausal Women Undergoing Chemotherapy for Early-Stage Breast Cancer: A Systematic Review and Meta-analysis. JAMA Oncol 2016; 2: 65-73.

12. Clough KB1, Goffinet F, Labib A, et al. Laparoscopic unilateral ovarian transposition prior to irradiation: prospective study of 20 cases. Cancer 1996; 77: 2638-45.

13. Revel A, Laufer N, Ben Meir A, et al. Micro-organ ovarian transplantation enables pregnancy: a case report. Human Reprod 2011; 26: 1097-1103.

14. Dolmans MM, Jadoul P, Gilliaux S, Amorim CA, Luyckx V, Squifflet J, Donnez J, Van Langendonckt A. A review of 15 years of ovarian tissue banking activities. J Assist Reprod Genet 2013; 30: 305-14.

15. Oktay K, Buyuk E, Veeck L, et al. Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet 2004; 363: 837-840.

16. Demeestere I, Simon P, Emiliani S, et al. Orthotopic and heterotopic ovarian tissue transplantation Human Reprod Update 2009;15: 649-665.

17. Richard A, Anderson W, Hamish B Wallace WH, et al. Ovarian tissue cryopreservation for fertility preservation: clinical and research perspectives, Human Reproduction Open Volume 2017, Issue 1, 29 March 2017.

18. Silber SJ, DeRosa M, Goldsmith S, et al. Cryopreservation and transplantation of ovarian tissue: results from one center in the USA. J Assist Reprod Genet 2018; 35: 2205-2213.

19. Gellert SE, Pors SE, Kristensen SG, et al. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. J Assist Reprod Genet 2018; 35: 561-570.

20. Jensen AK , Kristensen SG, Macklon KTet al. Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark. Hum Reprod 2015; 30: 2838–2845.

21. Kim S, Lee Y, Lee S, et al. Ovarian tissue cryopreservation and transplantation in patients with cancer. Obstet Gynecol Sci. 2018; 61: 431-442.

22. Fatum M., McVeigh E. Ovarian Tissue Cryopreservation pp 73-82. In: Mostafa Metwally M., Tin-Chiu Li, Reproductive Surgery in Assisted Conception. Springer, London, 2015.

23. Wallace WH, Smith AG, Kelsey TW, et al. Fertility preservation for girls and young women with cancer: population-based validation of criteria for ovarian tissue cryopreservation. Lancet Oncol. 2014; 15: 1129-36.

24. Meirow D, Ra'anani H, Shapira M, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril 2016; 106: 467-74.

25. Meirow D, Baum M, Yaron R, et al. Ovarian tissue cryopreservation in hematoloical malignancy: Ten years experience. Leuk & Lymph 2007; 48:1569-1576.

26. Dolmans MM, Jadoul P, Gilliaux S, et al. A review of 15 years of ovarian tissue banking activities. J Assist Reprod Genet 2013; 30: 305-14.

27. https://www.hta.gov.uk/policies/hfea-and-hta-joint-statement-ovarian-and-testicular-tissue-storage

28. National Institute for Health and Clinical Excellence. Fertility assessment and treatment for people with fertility problems. 2004.

www.rcog.org.uk/resources/Public/pdf/Fertility_summary.pdf

Human Reproduction Open, pp. 1–8, 2019
doi:10.1093/hropen/hoz016

human reproduction open

OPINION

Time to consider ovarian tissue cryopreservation for girls with Turner's syndrome: an opinion paper

Yadava Bapurao Jeve [1,*], Tarek Gelbaya[2], and Muhammad Fatum[3]

[1]Birmingham Fertility Centre, Birmingham Womens' Hospitals, Birmingham, UK [2]Leicester Fertility Centre, University Hospitals of Leicester, Leicester, UK [3]Nuffield Department of Women's Health and Reproduction, Oxford Fertility, University of Oxford, Oxford, UK

*Correspondence address: Birmingham Fertility Centre, Birmingham Womens' Hospitals, Edgbaston, Birmingham, UK.
E-mail: drybjeve@gmail.com

Submitted on March 19, 2019; resubmitted on April 29, 2019; editorial decision on May 10, 2019

ABSTRACT: Turner's syndrome (TS) is the most common sex chromosome abnormality in women. In addition to short stature and gonadal dysgenesis, it is associated with cardiac and renal anomalies. Due to rapid follicular atresia, the majority of women with TS suffer from primary ovarian insufficiency around puberty. Thus far, donor oocyte conception has been the key fertility option for these women. With advancing technology, ovarian tissue cryopreservation (OTCP) has emerged as a clinically justifiable option especially for pre-pubertal girls with cancer. Recently published results following the use of cryopreserved ovarian tissue are reassuring. It would be prudent to consider the extension of these technological and scientific advances to other conditions, such as TS, where accelerated follicular atresia is suspected. It is possible to obtain competent oocytes from cryopreserved ovaries of girls with TS provided the ovaries were preserved before ovarian failure. However, it is a complex decision whether and when to offer OTCP as a fertility preservation (FP) option for girls with TS. The rate of decline in fertility is variable in girls with TS and can be more complex in cases with mosaicism. On the other hand, OTCP has shown some promising results in patients with cancer, which can potentially be replicated in TS and other benign indications of patients at risk of premature ovarian failure. There are proven psychological and clinical benefits of FP. Thus, an argument could be made for offering OTCP to these patients to endow these girls with the option of having biological fertility using this innovative technology. Ethical, clinical and psychological dilemmas should be considered, discussed and addressed before considering such a novel approach. We believe that the time has come to start this discussion and open this avenue of FP for girls with TS.

Key words: Turner's syndrome / ovarian tissue cryopreservation / IVF / fertility preservation / premature ovarian failure

Introduction

Turner's syndrome (TS) is the most common sex chromosome abnormality of human females with an incidence of approximately 1 in 2000 female live births (Gravholt et al., 2017). Short stature and gonadal dysgenesis are two of the characteristic clinical features of the syndrome, together with a broad range of other phenotypic characteristics, which include an increased risk for heart and renal defects (Ford et al., 1959). The range of morbidities associated with the syndrome can have a profound effect on quality of life. Infertility and short stature are major concerns for women diagnosed with TS, influencing their psychosocial development (Sylven et al., 1991). Growth hormone treatment at a young age results in accelerated growth velocity and an increase in overall height, as compared to untreated girls (Baxter et al., 2007). Consequently, oestrogen replacement and fertility treatment are currently the mainstay of their endocrine management. A spontaneous puberty occurs in 5–10% of girls with TS, but only 2–5% reaches menarche with the possibility of achieving pregnancy (Hovatta, 1999). A recent study showed an overall 5.6% (27/480) prevalence of spontaneous pregnancies in women with TS. Most of these pregnancies occurred in women with mosaic TS and only 0.4% (2/480) spontaneous pregnancies were reported in women with a non-mosaic (X0) karyotype (Bernard et al., 2016). Due to the accelerated follicular atresia seen in TS patients, the majority of adolescent girls undergo ovarian failure prior to or around the time of puberty (Modi et al., 2003). Traditionally, donor oocyte conception has been the predominant fertility option

for such patients. Donor oocyte conception has its own limitations including various pregnancy complications (Jeve et al., 2016), difficulty in obtaining a suitable donor and psychological issues associated with the process (Bracewell-Milnes et al., 2016). Although technologically acquired parenthood may add a highly-desired dimension to their social identities, a sense of loss could persist in most of the women's personal identities (Hallebone, 1991). With recent technological advancements, fertility preservation (FP) may be successfully achieved using oocyte cryopreservation for pubertal girls and ovarian tissue cryopreservation (OTCP) for pre-pubertal girls (Grynberg et al., 2016). Although the exponential development of technology in the past few decades has led to promising results in the field of FP with OTCP, there are many unanswered questions about the clinical use of OTCP in TS patients. The purpose of this article is to examine the current and likely future status of OTCP for girls with TS.

Biological mechanism of follicular atresia

In TS ovaries, the premature ovarian failure is believed to be the result of follicular depletion. The mechanism and onset of follicular depletion has been widely studied in literature. Follicular atresia is a highly orchestrated and periodic process that results in destruction and elimination of follicles and oocytes from the ovary. Apoptosis is recognized as a key factor in atresia of antral follicles (Tilly et al., 1991) and is carried out by several molecular pathways, including mainly the B-cell lymphoma (Bcl-2) family, tumour necrosis factor (TNF), caspases and transforming growth factor beta-β proteins (Hussein, 2005). Apoptosis is regulated by genes such as Bcl-2 (pro-survival), Bax (pro-apoptotic) and cellular myelocytomatosis (c-Myc), which are expressed in granulosa cells of both foetal and adult ovaries, suggesting their possible role in atresia (Nandedkar and Dharma, 2001). The ovarian microenvironment and the interplay between pro-apoptotic and anti-apoptotic molecules play a significant role in folliculogenesis. Apoptosis was studied by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) in human foetal ovaries (Modi et al., 2003). TUNEL analysis of the TS (45X) ovaries revealed massive apoptosis of the oocytes. It was concluded that chromosomal defects, by some means, accelerates apoptosis that probably leads to gonadal dysgenesis later in life.

The unique chromosomal mark-up in TS is the presence of only one copy of the X chromosome. However, in 46 XX females, one copy of the X chromosomes is inactivated to achieve a balanced gene expression dosage between males (XY) and females (XX) (Berletch et al., 2011). Therefore, logically, the absence of one sex chromosome would not be predicted to have any deleterious effect. Nevertheless, this X inactivation is incomplete in persons with a normal karyotype; 15% of the genes on the silenced X chromosome escape inactivation and are expressed from both chromosomes (Berletch et al., 2011). Abnormalities associated with TS are thought to be caused by haploinsufficiency of genes that are normally expressed by both X chromosomes. Haploinsufficiency of multiple genes on both arms of the X chromosome (both X chromosomes remain active in germ cells), in addition to pairing failure during meiosis, leads to gonadal dysgenesis in TS (Davenport, 2010). X-linked inhibitor of apoptosis protein is one of the key intracellular pro-survival proteins and an intracellular modulator of the TNF-alpha death signalling pathway in granulosa cells (Xiao et al., 2001). Key genes determining ovarian reserve include oocyte-derived bone morphogenetic protein 15 (BMP15) as a critical signal promoting the growth and differentiation of ovarian follicles, and its action is intrinsically linked to oocyte-derived growth and differentiation factor 9 (GDF9). Alternations in the *BMP15* and *GDF9* genes are associated with different ovarian phenotypic abnormalities (Rossetti et al., 2014). The process of accelerated apoptosis in the aneuploid gonad starts already in foetal life (Modi et al., 2003) and continues throughout childhood; therefore, most of the girls with TS fail to achieve the spontaneous puberty. The overall incidence of spontaneous puberty in TS is reported to be 5–10% (Pasquino et al., 1997). Therefore, it would be sensible to discuss options of FP before accelerated apoptosis leaves no follicles in the ovaries.

Current status of FP through OTCP

The first case of FP using OTCP, in a young woman with mosaic TS, was reported in 2008 (Huang et al., 2008). It was proposed more than 10 years ago that the combination of ovarian tissue cryobanking and immature oocyte collection from the tissue followed by IVM and vitrification of matured oocytes represents a promising approach of FP for young women with mosaic TS. OTCP protocols and the evidence supporting its use are derived mainly from patients with cancer. OTCP involves laparoscopic removal of ovarian tissue. To obtain the best results for cryopreservation, it is beneficial to remove the ovarian cortex from the medulla, which helps extreme penetration of cryoprotectants into the cortical tissue (Fathi et al., 2011). As ovarian reserve is already low in girls with TS, the recommendation is to remove as much tissue as possible, typically an entire ovary (Oktay et al., 2016). This is followed by making small strips of ovarian tissue to allow the cryoprotectants to penetrate the tissue. Slow freezing in liquid nitrogen has been the main procedure for preserving the ovarian tissue (Silber, 2012). The standard protocol for ovarian cryopreservation is slow programmed freezing, using human serum albumin-containing medium and propanediol, dimethylsulphoxide or ethylene glycol as a cryoprotectant, combined with sucrose (Hovatta, 2005). When the woman is ready to attempt pregnancy, autotransplantation of the thawed pieces of ovarian tissues is performed. Autotransplantation of the ovarian tissue can be performed in the ovarian fossae beneath the pelvic peritoneum (Oktay and Karlikaya, 2000; Pacheco and Oktay, 2017). The first live birth after orthotopic autotransplantation of cryopreserved ovarian tissue was reported in 2004 (Donnez et al., 2004). In a recent meta analysis, 85.2% of women had restored endocrine function, a 57.5% (69 of 120) clinical pregnancy rate and a 37.7% (65 of 172) live birth rate and ongoing pregnancy rate were reported (Pacheco and Oktay, 2017). This suggests approximately one in three women attempting the ovarian tissue transplant being able to have at least one child. This data is mainly derived from OTCP for cancer patients in different age groups. A recently published retrospective case-control study of 15 girls and young women aged 5–22 years with TS showed evidence of follicles in 60% of the biopsies; however, a high rate of abnormal follicle morphology was noted. The author suggested that the benefits of OTCP may be limited to a highly selected group of TS mosaic patients (Mamsen et al., 2019). Cryopreservation of oocytes or ovarian tissue has been performed experimentally in more than 150 girls and adolescents with TS over the past 16 years (Schleedoorn et al., 2019). The efficacy is still unknown for this subgroup of patients due to the lack of follow-up data.

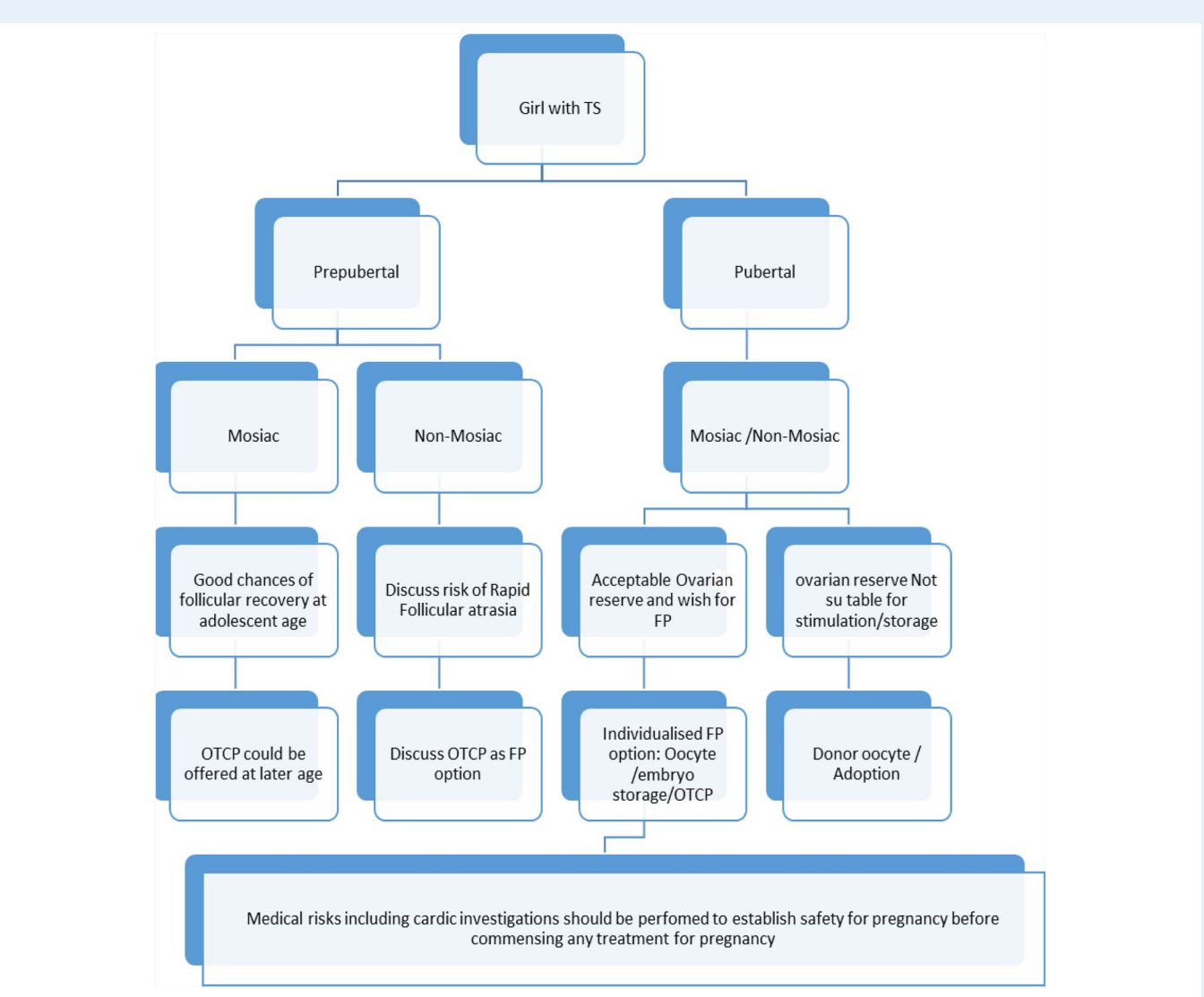


Figure 1 Fertility preservation (FP) options for girls with Turner's syndrome (TS). OTCP, ovarian tissue cryopreservation.

OTCP for girls with TS

Age to offer OTCP

The full biological development of ovaries is believed to be complete during the third trimester of pregnancy; therefore, it is biologically plausible that the ovarian tissue will be functional regardless of the age of the child that the tissue was cryopreserved. A confirmation of this notion was found by the report of the first live birth after an autograft of ovarian tissue that was cryopreserved before menarche, which was reported in 2015 (Demeestere et al., 2015). Recently, 84 children and eight ongoing pregnancies were reported following cryopreserved autologous ovarian tissue transplantation (Pacheco and Oktay, 2017), which includes adult and paediatric tissues. Chromosome abnormalities, such as TS, and trisomy 18 or 21, modify normal ovarian development by reducing the pool of available follicles and inhibiting follicular growth (Peters et al., 1978). Unless ovarian tissue is cryopreserved at a very early age, the success of this technology for girls with TS, especially 45X karyotype, may be thus predictably limited as a result of the accelerated follicular pool depletion. The chance of restoring fertility is related to the number and quality of follicles existing within the transplanted cortical tissue (Dolmans et al., 2009). It has been reported that primordial follicles can be found in ovarian tissue collected from girls (n = 57) with mosaic and non-mosaic TS up to the age of 17 years (Borgstrom et al., 2009). OTCP might be the only option for the paediatric and the pre-adolescent patients. Pre-pubertal girls with TS who are not timely assessed and considered for OTCP, could be missing the window of an opportunity for FP using OTCP, which could result in a TS generation without the real benefit of this technology. Figure 1 shows our suggested empirical approach for FP in girls with TS. Clinical decision making for FP is relatively clearer for pubertal girls with TS as it mainly comprises ovarian stimulation followed by oocyte or embryo vitrification. Patients with mosaic or non-mosaic TS with good ovarian reserve have the opportunity to undergo or not undergo FP depending on their ovarian reserve markers. There is a lack of conclusive data on ovarian reserve markers, such as antral

follicle count or anti-Müullerian hormone (AMH), for young pubertal girls (Lie Fong et al., 2012). The study evaluating serum AMH as a marker for ovarian reserve in young girls and adolescents reported that, as a screening test of premature ovarian failure in TS, the sensitivity and specificity of AMH <8 pmol/l was 96 and 86%, respectively (Hagen et al., 2010). Fifty-seven girls with TS aged 8–19.8 years were studied (Borgstrom et al., 2009). The author concluded that spontaneous puberty, mosaicism and normal hormone concentrations were significant but not exclusive prognostic factors for finding follicles in TS ovaries (Borgstrom et al., 2009). Therefore, the decision should be based on considerations of the complete clinical picture, desire of the individual and opinion of the team involved. It is prudent to discuss FP as soon as possible in both mosaic and non-mosaic TS. The patients or their parents or guardians should be made aware of all the related risks, benefits and future chances of fertility before making any decision for FP, in the best interest of the child. A provision should be made for supportive counselling for both patients and parents, to aid in decision making, and multidisciplinary team agreement before a child is subjected to laparoscopy and oophorectomy for OTCP.

Amount of tissue for OTCP

The amount of tissue to be taken for OTCP is another debatable issue open for discussion. Currently, there is no consensus on a standard operative technique for surgical ovarian cortical tissue removal in adult females, and there are limited published reports of the surgical approach and outcomes in the paediatric population (Corkum et al., 2017). Ovaries are often small in girls with TS, and therefore unilateral oophorectomy would be advantageous to only having several ovarian strips sampled and would render more tissue for preservation. In addition, unilateral oophorectomy carries a relatively low risk of bleeding. Laparoscopic unilateral oophorectomy for OTCP can be performed safely and as a day case procedure in children (Rowell et al., 2019). On the other hand, partial ovarian biopsy, with some ovarian tissue left remaining, offers a potential site for future implantation of cryopreserved tissue. The evidence evaluated from various age groups showed that at least $^{1}/_{2}$ to $^{2}/_{3}$ of the ovary should be taken from the anti-mesenteric surface of the ovary (Beckmann et al., 2016). It was found that premenopausal unilateral oophorectomy significantly reduces the age of menopause by 1.8 years, and patients or their guardians should be counselled accordingly (Rosendahl et al., 2017). Partial ovarian cortical biopsy or unilateral oophorectomy could be planned after careful counselling and an agreed strategy for the OTCP programme.

Clinical dilemma

Three prominent clinical concerns are debated while offering OTCP for girls with TS: insufficient evidence for the efficacy of this technology for TS, safety of women with TS carrying a pregnancy and risk of chromosomal anomalies in children. The current literature has reported promising results following OTCP and subsequent transplantation of preserved tissue as a new option of FP. More than 130 births have been reported worldwide following orthotopic transplantation of cryopreserved ovarian tissue (Beckmann et al., 2019). These results are mainly derived from FP before cancer treatment and largely from adult patients who had their ovarian tissue cryopreserved during adulthood. The age of patients at the time of tissue retrieval varied. A cohort study of 545 patients who had OTCP (mean age ± SD of 22.3 ± 8.8 years) reported 33% (7 out of 21) live births (Jadoul et al., 2017). A single-centre study reported on OTCP for 418 girls and adolescents aged younger than 15 years. Three hundred and thirteen patients had malignant diseases, and 105 had benign conditions. Recently three patients had auto-transplantation but no pregnancy has been reported yet (Poirot et al., 2019). A systematic review evaluating FP options in women with TS included 67 studies. The efficacy of different FP options is still unknown due to the lack of follow-up data (Schleedoorn et al., 2019). Another review reported on 1019 patients undergoing OTCP with ages ranging from 0.4 to 20.4 years, with 298 aged younger than 13 years. Eighteen patients underwent auto-transplantation of thawed ovarian cortical tissue that was cryopreserved before the age of 21 years, resulting in 10 live births (Corkum et al., 2019). An update paper on worldwide OTCP activity and on the Danish cohort reported a total of 93 children born, and 51% pregnancies were achieved by natural conception. The age range of patients at the time of OTCP who succeeded in having a live birth or ongoing pregnancy was 9–38 years (Gellert et al., 2018). Fertility outcome including successful natural or assisted conception for girls with TS who were pre-pubertal at the time of FP remains limited. This could be due to the fact that OTCP is a relatively new technology, and the pre-pubertal girls who had cryopreserved ovarian tissue are not yet ready to start fertility treatment. Ovarian tissue of girls with TS could be biologically different from that of girls with cancer, and therefore the success with OTCP may be different. It may be possible that despite OTCP women with TS will still have accelerated atresia after implantation of ovarian tissue. This is especially important for women who are planning natural conception. There could be a better chance if assisted conception is planned shortly after autologous implantation, but there is lack of evidence to support this opinion.

Regardless of whether conception is natural or assisted, using their own eggs or donated eggs, pregnancies in women with TS carry higher risks than the general population, mainly due to coexistent medical morbidities. Potential pregnancy complications are related to cardiac, renal and other medical conditions. In women with TS, the risk for aortic dissection or rupture during pregnancy may be 2% or higher (Practice comittee 2005). Maternal mortality in women with TS has been reported to be as high as 1–2%, which is 100 times greater than general population (Grynberg et al., 2016). Therefore, appropriate pre-pregnancy counselling and screening is recommended before pregnancies are planned by women with TS. Such a pregnancy should be managed as a high-risk pregnancy by a multidisciplinary team (Grynberg et al., 2016). It would be important to put a comprehensive pregnancy plan in place before embarking on any fertility treatment for women with TS. These obstetric risks are present irrespective of the origin of the gametes. Therefore, similar concerns could be debated for cryopreservation of the patients' oocytes or for donor eggs. If it is deemed relatively safe and clinically acceptable to use donor oocytes for conception for girls with TS, then the option of OTCP does not add any further risk for pregnancy. Additional increased obstetric risks are due to the donor origin of the oocytes. Donor oocyte pregnancy acts as an independent risk factor for pregnancy complications, including hypertensive disorders (odds ratio (OR), 3.92), small for gestational age (OR, 1.81) and preterm delivery (OR, 1.31), and these risks could possibly be eliminated by using autologous oocytes (Jeve et al., 2016). FP provides an opportunity for future fertility using a patient's own

gametes, should it be safe to carry a pregnancy in the future. For women who are at high risk of carrying a pregnancy because of cardiac or renal conditions, gestational surrogacy with preserved oocytes or ovarian tissue could be an option to have their own biological offspring. There is no conclusive data on the incidence of chromosomal abnormalities in biological children of women with TS. There is a possibility of meiotic non-disjunction in the oocytes of women with TS and chromosomal aberrations have been shown to be more common in children born after naturally conceived pregnancies (King et al., 1978; Swapp et al., 1989). It has been suggested that women with TS should be offered PGD, chorionic villous sampling or amniocentesis if fertilization of their own oocytes is successful (Karnis et al., 2003). The clinical dilemma becomes even more multifaceted if future research potential, such as oogonial stem cells (OSC), in-vitro activation (IVA) and oocyte–granulosa cell complexes are taken into consideration (Ghazal, 2013). However, these approaches are presently in their initial stages. OSC, identified in human ovaries (Kawashima and Kawamura, 2017), have been suggested to have the potential to develop into oocytes. The TS ovary contains a population of OSCs, but the ovarian stroma in TS does not support follicle formation. The IVA modality is based on fragmentation of ovarian tissue to disrupt Hippo signalling, with or without drug treatment to stimulate Akt signalling and resulting in IVA of folliculogenesis before autotransplantation (Kawashima and Kawamura, 2017).

Any clinical dilemma is often balanced on risk versus benefit ratio. OTCP has been demonstrated as a clinically viable and acceptable option of FP for children with cancer. We argue to extend its benefits to benign conditions, such as TS, as an option for future fertility.

Psychological dilemma

The psychological impact following the diagnosis of genetic conditions such as TS is under-reported. A significant psychological impact including social, behavioural and educational components is associated with TS (Saenger et al., 2001). Delayed initiation of sexual activities and an impaired sense of self-esteem in women with TS has been reported (McCauley et al., 1986a). Furthermore, it is thought that women with TS suffer from limited emotional arousal, high tolerance for adversity, unassertiveness and over compliance (McCauley et al., 1986b). This is compounded by the reports showing that premature menopause can impair sexual identity and sexual function along with a woman's well-being and achievement of life goals (Graziottin, 2007). However, it is not clear whether these psychological issues are due to some underlying genetic or hormonal influence on behaviour or due to the issues of short stature, physical anomalies and infertility (Saenger et al., 2001). A study has shown that infertility is the most frequently cited concern followed closely by short stature, regardless of age (Sutton et al., 2005). Infertility alone is associated with significant psychological distress (Lukse and Vacc, 1999). Even for persons who may have not planned to have children, the threat of infertility can result in a deep sense of loss and anger. With wider acceptance of growth hormone treatment, short stature is being successfully treated. Psychological wellbeing of the girls with TS deserves further attention beyond hormone treatment. Given the complexity of a TS diagnosis and the psychosocial impact of the condition, counselling on psychosocial issues and addressing concerns about the girls' daily life and future adult life needs to be integrated into standard paediatric endocrinologist care (Culen et al., 2017).

The inability to bear biological children was reported as the most prevalent and painful challenge endured by most of the adult women with TS (Sutton et al., 2005). Although the use of donor oocytes has been a widely used fertility option, evidence is limited on the psychological issues surrounding donor gamete conception (Bracewell-Milnes et al., 2016). Child-free living may be a reasonable choice for some women, but it would be helpful for girls and their parents to hear all life-plan and family-building options presented in a balanced manner. Girls in the peri-pubertal age group begin to realize the implication of TS, including reduced fertility potential. A clear guideline is required on how, when or what to discuss regarding fertility and potential FP options; and how to support them to accept their differences and empower them (Colindres et al., 2016).

The studies on the psychological and emotive effects of FP techniques are still scarce (Laganà et al., 2017). It can be argued that the primary benefit of OTCP is to promote autonomy by giving them the hope of having genetically concordant children in the future.

Ethical dilemma

Ethical dilemmas should be tested on the principle pillars of autonomy, beneficence, justice, non-maleficence and, additionally, confidentiality and honesty (Das and Sil, 2017). One of the top choices an individual makes in life is the opportunity to have one's own children. An individual's autonomy of choice, which is an essential foundation of a free society, should be respected (Carvalho et al., 2017). Unique ethical issues arise in the paediatric and adolescent population while applying these ethical principles. The moral and legal recognition of autonomy is achieved normally with informed consent. However, it would be difficult to establish autonomy in children in certain age groups especially when discussing future fertility and childbearing. It is commonly an easy decision when the child, parents and caring team agree on the best interest of the child but it would be difficult if there is a disagreement. The ESHRE Task Force on Ethics and Law recommended that the child's decision should be respected if the child is mature and understands the issues at stake. When the child is immature, the decision to cryopreserve (or not) may be taken by the parents or the guardians, unless it poses grave prejudice to the well-being/welfare of the child. The child's or adolescent's opinion should be sought when they are able to understand the circumstances (de Carvalho et al., 2017). The importance of preserving the possibility of having genetically related offspring in the future is generally recognized, and the parents will have to decide whether this benefit outweighs the current risk of intervention for their child (ESHRE Task Force on Ethics and Law, 2004). The decision of FP is complex enough for an adult deciding for themselves, but parents face additional decisional conflict and regret when making the decision for their child (Li et al., 2017). On the principle of beneficence and non-maleficence, meaning risk and benefit analysis, it is recommended that discussion should include the potential risks emanating from application of the technique weighed against the proposed but as yet unproven benefits. It is important to note that the promising data published so far is derived from the cancer population and various age groups at the time of cryopreservation. FP could be justified on medical indications, social grounds and on the prevention of the biopsychosocial impact of a procreating disability

(Carvalho et al., 2017). At the same time, the powerful desires for a biological child should be weighed against the risks of pregnancy in TS. Such desires may have biological roots, but it is reinforced by powerful social and cultural expectations about motherhood; these expectations should be satisfactorily questioned rather than uncritically accepted. The tissue storage period should be until the age at which it is considered acceptable for the tissue to be used for the achievement of a pregnancy, taking into account the welfare of the child and the risks to the pregnant mother (Carvalho et al., 2017). Further, consensus on posthumous reproduction is still required. A task force recommended that the parents do not have the right to decide about the (reproductive) use of the genetic material of their child after his/her death (ESHRE Task Force on Ethics and Law, 2004). All specialties present in the caring team (oncologists, paediatricians, reproductive specialists, psychologists/counsellors) should be heard during decision-making about the best procedure. Another important ethical aspect is the question of legal ownership and rights applying to the banked ovarian tissue. The legislation may vary in different countries or may be lacking in some countries. Parental consent should not be static and should be reviewed regularly with increasing involvement of the child where possible, especially as children become legally competent. These regulatory issues need to be addressed based on local and national guidance. Where appropriate, guidance could be requested from the local medical ethical committee. Although the ESHRE task force guidance provides a good ethical framework for cryopreserved ovarian tissue (ESHRE Task Force on Ethics and Law, 2004), there is a crucial role for a multidisciplinary Ethics Committee to guide clinical practice in individual circumstances (Voultsos et al., 2016).

Conclusions

Recent evidence has supported OTCP as one of the promising options for FP. Although there is no convincing evidence on fertility outcome for girls with TS, further studies are urged to evaluate the efficacy of OTCP in girls with TS. While waiting for the evidence to accumulate we feel that it is still important to discuss this option with children and their family. Current and future rapid development in the pertinent technologies would allow OTCP to be a successful and widely accepted method. However, young girls diagnosed with TS are faced with rapidly reducing fertility potential due to increased follicular depletion and atresia. Further delay in offering them OTCP may obviate their chances of FP. It could be argued that OTCP was offered to cancer patients a while ago despite lack of enough evidence at the time, which now is shown to be one of the successful methods. Therefore, the discussion on FP using OTCP for these girls should be started as soon as the diagnosis is established. When it comes to FP, all treatment options should be discussed along with their efficacy. Our duty is to fully inform patients about the various options, including OTCP, and enable them and their families to make an informed decision based on available evidence. At some point in their life, this may be their only option to preserve their fertility. In the present review, we have argued the case for offering OTCP to girls with TS after a well-informed discussion and counselling by the relevant multidisciplinary team. A multidisciplinary team should be actively involved in the decision-making, addressing all clinical, psychological, legal and ethical aspects while keeping the patient's own interests at the centre of the discussion.

Authors' roles

Y.J. contributed significantly in design, acquisition, analysis and interpretation of current evidence, along with drafting the article. T.G. contributed substantially by revising the manuscript critically and adding significant intellectual contents. M.F. contributed substantially in conception and design of the manuscript in addition to critically revising it with further addition of valuable contents.

Funding

None.

Conflict of interest

No conflict of interest.

References

Baxter L, Bryant J, Cave CB, Milne R. Recombinant growth hormone for children and adolescents with Turner syndrome. *Cochrane Database Syst Rev* 2007;1–34, Cd003887.

Beckmann MW, Dittrich R, Findeklee S, Lotz L. Surgical aspects of ovarian tissue removal and ovarian tissue transplantation for fertility preservation. *Geburtshilfe Frauenheilkd* 2016;**76**:1057–1064.

Beckmann MW, Lotz L, Toth B, Baston-Büst DM, Fehm T, Frambach T, Germeyer A, Goeckenjan M, Häberlin F, Henes M *et al*. Concept paper on the technique of cryopreservation, removal and transplantation of ovarian tissue for fertility preservation. *Geburtshilfe Frauenheilkd* 2019;**79**:53–62.

Berletch JB, Yang F, Xu J, Carrel L, Disteche CM. Genes that escape from X inactivation. *Hum Genet* 2011;**130**:237–245.

Bernard V, Donadille B, Zenaty D, Courtillot C, Salenave S, Brac de la Perriere A, Albarel F, Fevre A, Kerlan V, Brue T *et al*. Spontaneous fertility and pregnancy outcomes amongst 480 women with Turner syndrome. *Hum Reprod* 2016;**31**:782–788.

Borgstrom B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, Fridstrom M, Hovatta O. Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab* 2009;**94**:74–80.

Bracewell-Milnes T, Saso S, Bora S, Ismail AM, Al-Memar M, Hamed AH, Abdalla H, Thum MY. Investigating psychosocial attitudes, motivations and experiences of oocyte donors, recipients and egg sharers: a systematic review. *Hum Reprod Update* 2016;**22**:450–465.

Carvalho BR, Kliemchen J, Woodruff TK. Ethical, moral and other aspects related to fertility preservation in cancer patients. *JBRA Assist Reprod* 2017;**21**:45–48.

Colindres JV, Childress KJ, Axelrad M, McCullough LB, Shao Y, Macias C, Loveless J, Gunn SK, Bercaw-Pratt J, Sutton R *et al*. A multidisciplinary approach to puberty and fertility in girls with Turner syndrome. *Pediatr Endocrinol Rev* 2016;**14**:33–47.

Corkum KS, Laronda MM, Rowell EE. A review of reported surgical techniques in fertility preservation for prepubertal and adolescent females facing a fertility threatening diagnosis or treatment. *Am J Surg* 2017;**214**:695–700.

Corkum KS, Rhee DS, Wafford QE, Demeestere I, Dasgupta R, Baertschiger R, Malek MM, Aldrink JH, Heaton TE, Weil BR *et al*. Fertility and hormone preservation and restoration for female children and adolescents receiving gonadotoxic cancer treatments: a systematic review. *J Pediatr Surg* 2019;**S0022–3468**:30886–30888.

Culen C, Ertl DA, Schubert K, Bartha-Doering L, Haeusler G. Care of girls and women with Turner syndrome: beyond growth and hormones. *Endocr Connect* 2017;**6**:R39–R51.

Das NK, Sil A. Evolution of ethics in clinical research and ethics committee. *Indian J Dermatol* 2017;**62**:373–379.

Davenport ML. Approach to the patient with Turner syndrome. *J Clin Endocrinol Metab* 2010;**95**:1487–1495.

Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C, Delbaere A, Devreker F, Ferster A. Live birth after autograft of ovarian tissue cryopreserved during childhood. *Hum Reprod* 2015;**30**:2107–2109.

Dolmans MM, Donnez J, Camboni A, Demylle D, Amorim C, Van A, Pirard C. IVF outcome in patients with orthotopically transplanted ovarian tissue. *Hum Reprod* 2009;**24**:2778–2787.

Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B, van A. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. *Lancet* 2004;**364**:1405–1410.

ESHRE Task Force on Ethics and Law. Ethical considerations for the cryopreservation of gametes and reproductive tissues for self use. *Hum Reprod* 2004;**19**:460–462.

Fathi R, Valojerdi MR, Eimani H, Hasani F, Yazdi PE, Ajdari Z, Tahaei LS. Sheep ovarian tissue vitrification by two different dehydration protocols and needle immersing methods. *Cryo Letters* 2011;**32**:51–56.

Ford CE, Jones KW, Polani PE, De JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet* **273**:711–713.

Gellert SE, Pors SE, Kristensen SG, Bay-Bjørn AM, Ernst E, Yding Andersen C. Transplantation of frozen-thawed ovarian tissue: an update on worldwide activity published in peer-reviewed papers and on the Danish cohort. *J Assist Reprod Genet* 2018;**35**:561–570.

Ghazal S. Oogonial stem cells: do they exist and may they have an impact on future fertility treatment? *Curr Opin Obstet Gynecol* 2013;**25**:223–228.

Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, Lin AE, Mauras N, Quigley CA, Rubin K *et al*. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati international Turner syndrome meeting. *Eur J Endocrinol* 2017;**177**:G1–G70.

Graziottin A. Effect of premature menopause on sexuality. *Womens Health* 2007;**3**:455–474.

Grynberg M, Bidet M, Benard J, Poulain M, Sonigo C, Cedrin-Durnerin I, Polak M. Fertility preservation in Turner syndrome. *Fertil Steril* 2016;**105**:13–19.

Hagen CP, Aksglaede L, Sorensen K, Main KM, Boas M, Cleemann L, Holm K, Gravholt CH, Andersson AM, Pedersen AT *et al*. Serum levels of anti-mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Rurner syndrome patients. *J Clin Endocrinol Metab* 2010;**95**:5003–5010.

Hallebone EL. Non-genetic mothers and their own children: infertility and IVF donor birth. *Aust J Soc Issue* 1991;**25**:122–136.

Hovatta O. Pregnancies in women with Turner's syndrome. *Ann Med* 1999;**31**:106–110.

Hovatta O. Methods for cryopreservation of human ovarian tissue. *Reprod Biomed Online* 2005;**10**:729–734.

Huang JY, Tulandi T, Holzer H, Lau NM, Macdonald S, Tan SL, Chian RC. Cryopreservation of ovarian tissue and in vitro matured oocytes in a female with mosaic Turner syndrome: case report. *Hum Reprod* 2008;**23**:336–339.

Hussein MR. Apoptosis in the ovary: molecular mechanisms. *Hum Reprod Update* 2005;**11**:162–177.

Jadoul P, Guilmain A, Squifflet J, Luyckx M, Votino R, Wyns C, Dolmans MM. Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases. *Hum Reprod* 2017;**32**:1046–1054.

Jeve YB, Potdar N, Opoku A, Khare M. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis. *BJOG* 2016;**123**:1471–1480.

Karnis MF, Zimon AE, Lalwani SI, Timmreck LS, Klipstein S, Reindollar RH. Risk of death in pregnancy achieved through oocyte donation in patients with Turner syndrome: a national survey. *Fertil Steril* 2003;**80**:498–501.

Kawashima I, Kawamura K. Disorganization of the germ cell pool leads to primary ovarian insufficiency. *Reproduction* 2017;**153**: R205–r213.

King CR, Magenis E, Bennett S. Pregnancy and the Turner syndrome. *Obstet Gynecol* 1978;**52**:617–624.

Laganà AS, La VL, Rapisarda AMC, Platania A, Vitale SG. Psychological impact of fertility preservation techniques in women with gynaecological cancer. *Ecancermedicalscience* 2017;**11**:ed62–ed62.

Li N, Jayasinghe Y, Kemertzis MA, Moore P, Peate M. Fertility preservation in pediatric and adolescent oncology patients: the decision-making process of parents. *J Adolesc Young Adult Oncol* 2017;**6**: 213–222.

Lie Fong S, Visser JA, Welt CK, de YB, Eijkemans MJ, Broekmans FJ, Roes EM, Peters WH, Hokken-Koelega AC, Fauser BC *et al*. Serum anti-mullerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *J Clin Endocrinol Metab* 2012;**97**:4650–4655.

Lukse MP, Vacc NA. Grief, depression, and coping in women undergoing infertility treatment. *Obstet Gynecol* 1999;**93**:245–251.

Mamsen LS, Charkiewicz K, Anderson RA, Telfer EE, McLaughlin M, Kelsey TW, Kristensen SG, Gook DA, Ernst E, Andersen CY. Characterization of follicles in girls and young women with Turner syndrome who underwent ovarian tissue cryopreservation. *Fertil Steril* 2019;**S0015–0282**:30081–30080.

McCauley E, Ito J, Kay T. Psychosocial functioning in girls with Turner's syndrome and short stature: social skills, behavior problems, and self-concept. *J Am Acad Child Psychiatry* 1986a;**25**:105–112.

McCauley E, Sybert VP, Ehrhardt AA. Psychosocial adjustment of adult women with Turner syndrome. *Clin Genet* 1986b;**29**:284–290.

Modi DN, Sane S, Bhartiya D. Accelerated germ cell apoptosis in sex chromosome aneuploid fetal human gonads. *Mol Hum Reprod* 2003;**9**:219–225.

Nandedkar TD, Dharma SJ. Expression of bcl (xs) and c-myc in atretic follicles of mouse ovary. *Reprod Biomed Online* 2001;**3**:221–225.

Oktay K, Bedoschi G, Berkowitz K, Bronson R, Kashani B, McGovern P, Pal L, Quinn G, Rubin K. Fertility preservation in women with Turner syndrome: a comprehensive review and practical guidelines. *J Pediatr Adolesc Gynecol* 2016;**29**:409–416.

Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. *N Engl J Med* 2000;**342**:1919.

Pacheco F, Oktay K. Current success and efficiency of autologous ovarian transplantation: a meta-analysis. *Reprod Sci* 2017;**24**: 1111–1120.

Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Spontaneous pubertal development in Turner's syndrome. Italian study Group for Turner's syndrome. *J Clin Endocrinol Metab* 1997;**82**:1810–1813.

Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab* 1978;**7**:469–485.

Poirot C, Brugieres L, Yakouben K, Prades-Borio M, Marzouk F, de G, Pacquement H, Bernaudin F, Neven B, Paye-Jaouen A *et al.* Ovarian tissue cryopreservation for fertility preservation in 418 girls and adolescents up to 15 years of age facing highly gonadotoxic treatment. Twenty years of experience at a single center. *Acta Obstet Gynecol Scand* 2019;**S0015–0282**:30081–30080.

Rosendahl M, Simonsen MK, Kjer JJ. The influence of unilateral oophorectomy on the age of menopause. *Climacteric* 2017;**20**: 540–544.

Rossetti R, Cacciatore C, Persani L, Di E, Fabre S. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. *Hum Reprod Update* 2014;**20**:869–883.

Rowell EE, Corkum KS, Lautz TB, Laronda MM, Walz AL, Madonna MB, Lockart BA, Reynolds M. Laparoscopic unilateral oophorectomy for ovarian tissue cryopreservation in children. *J Pediatr Surg* 2019;**54**:543–549.

Saenger P, Wikland KA, Conway GS, Davenport M, Gravholt CH, Hintz R, Hovatta O, Hultcrantz M, Landin-Wilhelmsen K, Lin A *et al.* Recommendations for the diagnosis and management of Turner syndrome. *J Clin Endocrinol Metab* 2001;**86**:3061–3069.

Schleedoorn MJ, van der A, Braat DDM, Peek R, Fleischer K. To freeze or not to freeze? An update on fertility preservation in females with Turner syndrome. *Pediatr Endocrinol Rev* 2019;**16**:369–382.

Silber SJ. Ovary cryopreservation and transplantation for fertility preservation. *Mol Hum Reprod* 2012;**18**:59–67.

Sutton EJ, McInerney-Leo A, Bondy CA, Gollust SE, King D, Biesecker B. Turner syndrome: four challenges across the lifespan. *Am J Med Genet A* 2005;**139a**:57–66.

Swapp GH, Johnston AW, Watt JL, Couzin DA, Stephen GS. A fertile woman with non-mosaic Turner's syndrome. Case report and review of the literature. *Br J Obstet Gynaecol* 1989;**96**:876–880.

Sylven L, Hagenfeldt K, Brondum-Nielsen K, von B. Middle-aged women with Turner's syndrome. Medical status, hormonal treatment and social life. *Acta Endocrinol* 1991;**125**:359–365.

Tilly JL, Kowalski KI, Johnson AL, Hsueh AJ. Involvement of apoptosis in ovarian follicular atresia and postovulatory regression. *Endocrinology* 1991;**129**:2799–2801.

Voultsos P, Raikos N, Vasileiadis N, Spiliopoulou C, Tarlatzis B. Ethico-legal issues related to ovarian tissue transplantation. *Med Sci Law* 2016 Oct;**56**:293–304.

Xiao CW, Ash K, Tsang BK. Nuclear factor-kappaB-mediated X-linked inhibitor of apoptosis protein expression prevents rat granulosa cells from tumor necrosis factor alpha-induced apoptosis. *Endocrinology* 2001;**142**:557–563.

Study Title: **In Vitro Culture and Maturation of Ovarian Tissue and Follicles: Follicle microenvironment engineering as a tool to study folliculogenesis and provide fertility preservation.**

Short title: Methods of ovarian tissue culturing for fertility preservation

Ethics Ref 14/SC/0041

Date and Version No: 24 February 2014 V1.1

Chief Investigator:	Muhammad Fatum, Clinical Research Fellow, University of Oxford, Nuffield Department of Obstetrics & Gynaecology, Institute of Reproductive Sciences, Oxford Business Park North, Oxford, OX4 2HW. Tel 01865-782-876
Investigators:	Prof Z. F. Cui, Director, Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, Parks Road OX1 3PJ Tim Child, Co-Director, Oxford Fertility Unit and Senior Clinical Fellow, University of Oxford
Sponsor:	University of Oxford
Funder :	BBSRC Grant
Signature:	The approved protocol should be signed by author(s) and/or person(s) authorised to sign the protocol

There are no conflicts of interest declared.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

1. SYNOPSIS

Study Title	**In Vitro Culture and Maturation of Ovarian Tissue and Follicles: Follicle Microenvironment Engineering as a tool to study folliculogenesis and provide fertility preservation**	
Short title	Methods of ovarian tissue culturing for fertility preservation	
Study Design	Bench research using donated human tissue samples	
Study Participants	Females aged 6-43 having clinically indicated ovarian tissue cryopreservation Women aged 18-43 having planned ovarian surgery or sterilisation	
Planned Sample Size	Up to 50	
Planned Study Period	5 years	
	Objectives	**Endpoints**
Primary	To determine a successful in vitro culturing system of early stage human follicles.	Number of follicles that survive in vitro during a certain period of time
Secondary	To identify follicular development and maturation using this system. To further identify factors involved in the in vitro maturation of follicles.	Proteomic, metabolomic and genomic studies to determine the success of the *in vitro* culture – measured by: • The increase in size of the follicles • Variations in the morphology of the follicles • Measuring numbers of follicles which form an antrum and time taken for antrum to develop • The concentration of hormones secreted by the follicles.

2. ABBREVIATIONS

CI	Chief Investigator
CRF	Case Report Form

CTRG	Clinical Trials & Research Governance, University of Oxford
GCP	Good Clinical Practice
GP	General Practitioner
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IVF	In Vitro Fertilisation
IVM	In Vitro Maturation
NHS	National Health Service
NRES	National Research Ethics Service
OCTE	Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGD	Three dimensional (3D) scaffold and adhesion motif
SOP	Standard Operating Procedure

3. BACKGROUND AND RATIONALE

3.1. Overview

For female adolescents and young adults, the increasing probability of surviving a cancer diagnosis combined with the expectation and desire for reproductive options following cancer remission has fuelled a growing need for fertility sparing techniques.

Currently there are several methods available to women hoping to preserve their fertility before cancer therapy. As it has been used most often, traditional hormone stimulation and in vitro fertilization (IVF) followed by embryo cryopreservation is the most successful approach to preserve fertility. More recently live births have also been achieved with cryopreserved oocytes harvested before cancer treatment. Both methods require a delay in cancer treatment and use hormonal stimulation that might be deleterious in some patients. Ovarian tissue cryopreservation followed by autotransplantion is a promising fertility preservation approach that can usually be performed immediately without hormonal stimulation. Worldwide, a dozen live births have been reported thus far as a result of auto-transplanting

frozen/thawed ovarian tissues(Donnez, Silber et al. 2011). Despite these promising findings, transplantation of cryopreserved tissue carries the risk of re-introducing cancer cells into the patient. Thus, the in vitro maturation (IVM) of immature follicles derived from ovaries collected prior to commencement of cancer treatment is an important direction for new fertility preservation approach as it avoids the need for auto grafting and prevents the possibility of cancer cells reseeding.

In this proposal we aim to study and improve the existing early-stage follicle culture protocol by the use of three dimensional (3D) scaffold and adhesion motif (RGD). These 3D culture systems can preserve the follicle architecture and maintain the cell-cell and cell-matrix signalling lost in two-dimensional attached follicle culture systems. Maintaining the follicular structure while monitoring and manipulating the hormonal and biochemical environment through the perfusion system will allow the development of controllable systems to investigate and improve the biological and molecular processes underlying follicle growth and maturation. Advances in our understanding of in vitro culturing and maturation of follicles may also be applied to provide a fertility preservation option for women at risk of premature ovarian insufficiency as well as facilitating research in folliculogenesis.

3.2. Fertility Preservation in Cancer Patients

In recent years cryopreservation of human ovarian tissues has emerged as a promising alternative for fertility preservation in pre-menarchal and adolescent females, as well as for adult women prior to chemo/radiotherapy. The prognosis and survival rates of cancer patients have improved tremendously as a result of recent advances in cancer treatment, particularly in childhood cancers, and attention is now being directed towards quality of life and the long-term gonadotoxic side effects of chemotherapy or radiotherapy. Given that 1 in 46 females between birth and age 39 are diagnosed with cancer(American Cancer Society 2012) many women are potentially affected. The incidence of ovarian failure is dependent on the type and dose of chemotherapy agents, and the age of the patient. Currently, there are several modalities for fertility preservation for different age groups:

Post-pubertal women

Post pubertal women requesting fertility preservation prior to chemotherapy or radiotherapy may be offered any of the following options:

3.2.1. Embryo cryopreservation

This is currently the only widely accepted and well-established option to preserve fertility, during which patients undergo a stimulated IVF cycle with subsequent retrieval of mature oocytes prior to the

initiation of chemo/radiotherapy. Harvested oocytes are then fertilized with sperm produced by husband, partner or donor, and the resulting embryos are cryopreserved. This technology is in routine clinical use in IVF units throughout the world on a daily basis. An evolving newer strategy is obtaining immature oocytes in non-stimulated cycle for in vitro maturation of oocytes and subsequent fertilization, and embryo freezing.

The advantages of embryo cryopreservation modality are its worldwide availability and its proven and established pregnancy success rates. Disadvantages include the need for hormonal stimulation of 2-4 weeks (which may defer and delay the anticancer treatment), its costs (£4000-5000), the need for a partner/husband or the decision of using donor sperm, and the relatively limited number of embryos produced and stored from a single stimulation cycle. It is not a viable option for pre-menarchal or adolescent girls, single women unable/unwilling to use donor sperm and women who are unable to undergo an *in vitro* fertilization cycle due to the need to undergo immediate anticancer treatment. In addition, the IVF procedure, especially hormonal stimulation, causes discomfort and increases the risk for OHSS, ovarian, breast and endometrium cancer, and stroke (Gilchrist et al., 2011). Additionally, there are ethical issues regarding embryo storage and usage.

3.2.2. Oocyte cryopreservation

In this modality, the patient undergoes a stimulated IVF cycle and subsequently the harvested oocytes are cryopreserved for later thawing out and fertilization. This technology can be an option for single female patients who are not in a stable relationship and not willing to opt for sperm donation. Because of the large size and more complex and fragile nature of the oocyte, oocyte freezing has been technically difficult with limited proven success rates. The introduction of rapid vitrification is promising, as it has been shown to have much elevated oocyte survival rates. However, the issues with the hormonal stimulation described above remain.

A newer strategy of oocyte preserving involves obtaining immature oocytes from unstimulated ovaries. These immature oocytes, which are smaller-sized, less complicated structurally and metabolically less active than mature oocytes, are either subsequently frozen or cultured in an *in vitro* system and cryopreserved as mature oocytes. The merits of this option include the sparing of the ovulation induction process; as a result, it can be performed immediately, avoiding the delay of chemo/radiotherapy and has lower risks related to exposure of high oestradiol levels. In addition, it requires less patient monitoring and reduces the associated costs. The challenge is the *in vitro* maturation process.

3.2.3. Ovarian tissue cryopreservation

In this approach an operative procedure (usually laparoscopy, rarely laparotomy) is performed, prior to commencement of anticancer treatment, to harvest ovarian tissue. This is cryopreserved and is followed by auto-transplantation later in life i.e. following recovery from the cancer treatment and when patient is ready to proceed with fertility treatment. The so called autografting of the cryopreserved tissue involves transplanting the ovarian tissue back into the patient immediately after thawing, either to the ovarian fossa (orthotopic graft) or to a different site (heterotopic graft). With autografting, immature follicles and oocytes would mature in *vivo* (thus obviating the need for exogenous gonadotropin stimulation) or ovulation induction treatment or IVF cycle may be needed. This treatment is still considered experimental, albeit a promising one for fertility preservation. An increasing number of pregnancies are being reported and thus far approximately 13 live births have been reported using this technology (Donnez, Silber et al. 2011).

As with immature oocyte cryopreservation, this approach can be performed immediately, often without hormonal stimulation. It may be offered to women undergoing aggressive chemotherapy/radiotherapy with a high likelihood of gonadotoxicity. This modality may be suggested as well for fertility preservation in young women to allow future childbearing through assisted conception without the risk of age related loss of fertility, the so called- social indication. There are several points to be taken into account when considering this approach and to tailor treatment to the individual concerned: the reproductive age, the chance of survival and the relative risk of subfertility related to the chemotherapy versus the possibility of the re-introduction of cancer cells and other clinical considerations.

3.2.4. Other options for fertility preservation

Ovarian suppression prior to chemotherapy

The use of GnRH analogues for ovarian suppression is controversial, and with no hitherto established or well-proven value. Its use should be considered in well-designed experimental protocols.

Oophoropexy prior to radiotherapy:

This modality includes an operative procedure, mainly laparoscopy, to move ovaries out of the pelvic radiotherapy field. It would be appropriate for only a very few well-defined patients.

Pre-pubertal children:

Ovarian tissue cryopreservation, though still considered as an experimental approach, is the only available fertility preservation approach that can be offered for premenarcheal/ pre-pubertal girls.

Currently, this technique is available in only a limited number of fertility centres in several countries. It has been used in children as young as 2.7 years of age.

In summary, ovarian tissue preservation is a feasible option to preserve ovarian function and possibly fertility in children and young women before gonadotoxic chemotherapy and/or radiotherapy. However, further research is needed to assess the clinical effectiveness of ovarian cryopreservation, to optimise the technique and to define clear indications.

3.3. Challenges with Ovarian Tissue Preservation

Restoration of ovarian function with hormone production and follicular growth has been observed after transplantation of frozen–thawed ovarian tissue (Oktay, Newton et al. 2000; Radford, Lieberman et al. 2001; Oktay, Buyuk et al. 2004; Tryde Schmidt, Yding Andersen et al. 2004; Schmidt, Andersen et al. 2005; Rosendahl, Loft et al. 2006; Donnez, Squifflet et al. 2008), and more than a dozen of pregnancies (Donnez, Dolmans et al. 2004; Meirow, Levron et al. 2005; Demeestere, Simon et al. 2006; Demeestere, Simon et al. 2007; Meirow, Levron et al. 2007; Andersen, Rosendahl et al. 2008) have been reported to date.

Nevertheless research efforts are still needed to develop and optimize grafting procedures; a large percentage of grafted follicles are lost as a result of post grafting ischemia and reperfusion-induced damage (Newton et al., 1996; Aubard et al., 1999; Donnez et al., 2006b). In addition, some studies have demonstrated the risk of possibly transmitting malignant cells present in the cryopreserved tissue back to patient's body (Shaw et al., 1996; Meirow et al., 1998, 2008). Thus, an important direction for research is into ways to avoid the need for autografting, such as the *in vitro* maturation of immature follicles derived from ovaries collected prior to commencement of cancer treatment. So far, most cases utilising *in vitro* maturation (IVM) have been from patients with polycystic ovaries (PCO) (Tan and Child 2002; Gremeau, Andreadis et al. 2012). This is mainly because of the greater number of small follicles in PCO ovaries. This technology may similarly be suggested for patients with a high number of antral follicles. A successful *in vitro* culture system for ovarian tissue would then enable the growth and maturation of follicles and oocytes which could be subsequently fertilized to produce viable embryos.

3.4. In vitro culture and maturation of follicles

In order to avoid the risk of transmitting cancer cells as well as other technical challenges of grafting including long term biocompatibility, matrix degradation vascularization, and integration with existing tissue (Kim and Mooney 1998; West, Xu et al. 2007), isolated follicles could be grown *in vitro* to achieve

in vitro maturation of oocytes followed by fertilization and embryo transfer. The IVM procedure would also allow the assessment of follicular quality after cryopreservation by the direct monitoring of follicles during the culture period (Abir, Roizman et al. 1999), which could also help to assess the factors involved in the folliculogenesis in humans.

The first human oocyte IVM was performed by Edwards in 1965 (Edwards 1965). The purpose of the *in vitro* human follicle growth (IVFG) system is to mimic the *in vivo* process by providing follicles with appropriate growth factors and hormones, in the correct amount and at the right time, to enable growth of the follicle and oocyte whilst maintaining the essential connections between somatic cells and the oocyte (called transzonal projections (TZPs)). (Anderson and Albertini 1976; Albertini and Barrett 2003; Xu, Barrett et al. 2009). At the moment IVM is regarded as an adjunct procedure to IVF but successful *in vitro* culturing and maturation of the ovarian tissue or isolated follicles to produce mature follicles and oocytes, would provide a safe fertility preservation option.

Currently there are two standard clinical protocols for IVM (Gilchrist, Smitz et al. 2011). First, the cumulus-oocyte complex is removed when the woman's endometrium thickness is at least 5 mm. The complex removed from the meiotically inhibiting environment is spontaneously matured to the metaphase II stage in 36 hours. The other method involves a large bolus of Human Chorionic Gonadotrophin (HCG) administration, to initiate meiotic resumption, 36 hours prior to oocyte retrieval. In this case IVM time is significantly reduced to 24 hours. The In vitro culture systems can also be categorized into two approaches. In the first approach isolated follicles are placed on two-dimensional surface and permitted to attach and spread on the surface. The second one is the three dimensional intact follicle approach in which follicles do not adhere to a surface but maintain their architecture and the cell-cell and cell-matrix interactions which are critical regulators of follicle development (West et al., 2007).

These current clinical IVM methods only support late stage pre-ovulatory follicles. However, as the procedures depend on the hormonal regulation and the availability of antral follicles, they are not yet an option for pre-pubertal children and cancer patients who require urgent treatment. The ability to culture immature follicles will be invaluable for fertility preserving technologies. Primordial follicles are the most abundant follicle stage in the ovary and present at all ages. They are also less susceptible to cryogenic and cancer therapy damage.

Therefore, IVM for pre-antral follicles should be developed, although to date the culture of early stage (primordial or primary) follicles to the antral stage has yet to be fully elucidated (Galdones, Shea et al. 2011) and only a few studies have been performed and published (Roy and Treacy 1993; Oktay, Nugent

et al. 1997; Abir, Roizman et al. 1999; Abir, Fisch et al. 2001). However, although the in vitro culture of isolated small pre-antral follicles (30–50 µm) from human ovaries has not yet yielded satisfactory results, samples from fresh and frozen–thawed ovarian tissue have been shown to survive in vitro for up to 24 hours (Abir, Roizman et al. 1999; Abir, Fisch et al. 2001) in collagen gel.

At the moment, two step approaches seem to be promising in the development of a method for the culture of primary follicles. Because of the different requirements of the primary and secondary follicles, two separate systems were developed to support each development stage. First, primary follicles in *ex vivo* ovarian tissue were cultured to secondary follicles. Then the secondary follicles were collected and cultured in an encapsulated system. Murine fetal ovaries and cryopreserved ovaries have been cultured successfully using this two-step method.

Unfortunately, another challenge of in vitro human follicle culture is that most IVM studies have previously been done in rodents where the terminal follicle size is smaller and the culture period is shorter. The increased volume means the follicles are more susceptible to sheer and diffusion limitation. The human ovary also contains more fibrous stroma which makes follicle collection difficult. Telfer and colleagues tried to culture human preantral follicles using the two step culture system, but the follicles could maintain their structure only up to 4 days (Telfer, McLaughlin et al. 2008). However, human secondary follicles could be cultured to the antral stage in the presence of activin.

Animal studies have shown that the major problem in early stage IVM is how to maintain the oocyte development competence in the artificial environment. Despite all attempts to manipulate the culture conditions, the differences between cellular environment in standard 2D cell culture system and the real physiological environment usually exist and are significant, which results in the deviated cellular behaviour. For example, more abnormal chromosome configurations and disturbed methylation pattern on the gene H19 are observed in IVM oocyte compared to oocyte matured *in vivo* under ovarian stimulation (Borghol, Lornage et al. 2006; Li, Feng et al. 2006). As a result, these oocytes are likely to fail to mature and result in high miscarriage rate of IVM derived embryos.

Most IVM researchers have tried to improve IVM conditions by optimizing culture medium formula. Pre-IVM treatment with cyclic adenosine monophosphate (cAMP) modulating agents and addition of, for example, FF-MAS, oocyte secreting factors, protein kinase c activator, have shown to enhance the embryo yield and the fetal survival significantly see Gilchrist for reviews (Gilchrist, Smitz et al. 2011). Nevertheless, Abir et al. reported that isolated follicles could only grow in a supporting matrix i.e. a 3D system(Abir, Nitke et al. 2006), possibly due to the preservation of intercellular interactions between granulosa cells (GCs) and the oocyte in this system and the provision of optimal support for the fragile

isolated follicles, similar to the ovary itself.

3.4.1. 3D scaffold

In the ovary, primordial and primary follicles are localized to the rigid part of the ovary and migrate to the less rigid part while growing. The presence of 3D scaffold and its rigidity are sensed through cellular contact that subsequently triggers various signalling cascades for cell survival and differentiation. Subsequently, cells survival period and tissue integrity are enhanced.

Two-dimensional cultures have been revealed as insufficient for sophisticated cell culture. Over time the structure of the spherical follicle is disrupted as it flattens and the loss of follicular structure releases denuded oocytes. The degree of disruption increases with the terminal size of the follicles. 2D follicle tissue culture also leads to granulosa cell proliferation, migration from the oocyte, and subsequent flattening of the oocyte (Pangas, Saudye et al. 2003).

Morphophysiological observations suggest that the suitable conditions for *in vitro* oocyte maturation maintain its cellular environment and well defined extracellular matrix structure in 3D organization (Torre, Faustini et al. 2007). Many aspects of oocyte growth and development are believed to be regulated by interactions with adjacent GCs (Murray, Gosden et al. 1998; Reynaud, Cortvrindt et al. 2000) and this is probably why most studies are performed with pre-antral follicles enclosed in ovarian tissue (Hovatta, Silye et al. 1997; Hovatta, Wright et al. 1999; Carlsson, Scott et al. 2006).

Cell encapsulation is the technique whereby a pool of live cells is entrapped within a semi permeable membrane. Since the first publication by Chang et al in 1964 (Chang 1964), bioencapsulation has gained interest from researchers. The first application of cell encapsulation in reproduction was made by Nebel et al. who devised a technique for bovine sperm encapsulation in calcium alginate and polyamines (Nebel, Bame et al. 1985). Later, Pangas et al. developed an alginate beads 3D culture system designed for cumulus/oocyte complexes (Pangas, Saudye et al. 2003); such technology yielded good results in terms of structural development and meiosis resumption for murine ovary. 3D culture scaffold will slow the structural deterioration of the spherical follicle by giving mechanical support (Smitz and Cortvrindt 1998).

3.4.2. Alginate Hydrogel

Bioactive material such as collagen and Matrigel would appear to be excellent scaffold for in vitro follicle culture however, since the matrices are bioactive, some degree of cell migration from the follicle and

enzyme activity can lead to changes in the follicle structure and follicular damage. Inert 3D matrix such as alginate or polyethylene glycol (PEG) has proven to be a well characterized and customizable tool for *in vitro* culture.

Alginate is a nontoxic polysaccharide composed of repeating units of mannuronic (M) and glucuronic acids (G) that may be reversibly linked by exposure to divalent cations such as calcium (Ca^{2+})(Pangas, Saudye et al. 2003; Torre, Faustini et al. 2007). It is permeable to small molecules such as glucose and oxygen and its permeability can be easily tailored to suit different applications. The alginate encapsulation procedure does not damage the ovarian cells or cellular junctions. It results in a surrounding alginate matrix that maintains the positions of the granulosa cells within the oocyte; hence preserving the granulosa cell–oocyte interactions in the presence of appropriate growth and differentiation factors (Pangas, Saudye et al. 2003). Also, oocytes grown in vitro can be readily retrieved from the matrix for further uses by the addition of a chelating agent such as EDTA or sodium citrate. Further uses could include in vitro fertilisation. According to the properties described above, alginate hydrogel should be suitable for *in vitro* culture of isolated follicles. Its porous structure allows diffusion of hormones and other proteins that are essential for follicular development. Indeed, alginate is one of the most widely used biomaterials for microencapsulation because of its biocompatibility, high affinity to water and ability to form hydrogels under very mild conditions (Smidsrod and Skjak-Braek 1990; Draget, Skjak-Braek et al. 1997; Amsden and Turner 1999; West, Xu et al. 2007).

Moreover, alginate scaffold can be functionalised for specific use. Small proteins and functional groups can be attached to the alginate scaffold by chemical modification. The rigidity and mechanical properties of the alginate can be altered by the ratio of G:M monomer and calcium concentration in the linking solution. The dynamic characteristics of alginate scaffold make it a suitable material for robust dynamic *in vitro* follicle culture.

A fluid core alginate capsule can support human oocyte maturation well with 90.3% success rate after 48 hr. Recently, another 3D alginate hydrogels system has been successfully applied to in vitro culture of isolated mouse (Pangas, Saudye et al. 2003; Kreeger, Fernandes et al. 2005; Kreeger, Deck et al. 2006; Xu, Kreeger et al. 2006; Xu, West et al. 2006; West, Xu et al. 2007) and rat (Heise, Koepsel et al. 2005) follicles. Mouse follicle encapsulated in alginate beads culture in 100 uL growth medium (a-MEM-0.3% BSA, fetuin 1 mg/mL) for 10 days grew as intact spheres of cells within the alginate bead. At this stage, the oocytes showed no signs of degeneration, had cortical granules around the periphery, and contained an intact zona pellucida with numerous microvilli projecting from the oocyte. The granulosa cells surrounding the oocyte were organized, spherical and had few vacuoles. No morphological evidence of cellular apoptosis was noted (Pangas, Saudye et al. 2003). Follicles cultured in alginate produced

fertilizable oocytes and live mice have been born from these (Pangas, Saudye et al. 2003; Xu, Kreeger et al. 2006). However, alginate exhibits minimal cellular interactions with mammalian cells, and thus likely provides only mechanical support.

3.4.3. Adhesion motif RGD

Along with the signalling molecules between cells in the follicle, the interaction between follicle cells and the surrounding extracellular matrix (ECM) also appear to contribute to follicle fate (Irving-Rodges et al, 2009).Most of the inert scaffolds lack interaction with the cells. They are coated with cell surface adhesive proteins such as collagen, laminin and fibronectin to enhance cell attachment. However, these proteins are usually derived from animals so they may be contaminated with pathogen or trigger the immune response. Also, since they are susceptible to denaturation and degradation, they need to be replenished constantly. What is more the binding of large protein molecules onto scaffold backbone molecules such as alginate is not easy technically. To overcome these drawbacks, immobilized peptides are presented as the cell recognition motifs instead. ECM usually contains several protein motives to interact with specific receptors. The smaller peptide motif is more stable and can be packed at higher density.

RGD peptide is a cell recognition motif that consists of Arginine (R), Glycine (G), Aspartic acid (D). It is recognized by cell surface receptors of the integrin family. Integrins are the major receptors mediating adhesion to the extracellular matrix. Following ligand binding, conformational changes of integrins induce the recruitment of multiple signalling and scaffolding proteins that connect integrin tails to the actin cytoskeleton and permit activation of signalling pathways regulating cell proliferation, apoptosis, differentiation, and migration. In the ovary, extracellular matrix components present in the follicular basement membrane, around follicular cells, and in the follicular fluid and the role of integrins in the regulation of follicular development is strongly suggested (Monniaux, Huet-Calderwood et al. 2006). Smitz et al. reported that the presence of an extracellular matrix enhances follicle survival, especially after 10 days in mouse follicle culture and 14 days in sheep follicle culture (Smitz and Cortvrindt 1998), but there is no significant difference in follicle diameters with ECM culture compared to non-ECM culture.

Kreeger et al showed that encapsulation and culture of follicles with RGD-modified alginate significantly increased growth of secondary follicles and improved meiotic competency rates of oocytes compared with follicles cultured in alginate alone (Kreeger, Deck et al. 2006).

3.4.4. Perfused system

Follicles at different stages require different culture conditions. Consequently, well defined and well controlled culture environment will be the key to the success of in vitro follicle culture and this can only be achieved in a dynamic perfused culture system. In the conventional static culture environment all parameters in the culture medium change with time depending on metabolic rate and local conditions. Perfused technology, where culture media is pumped and flows through the culture at a pre-set manner, not only allows the better transportation (i.e. overcome diffusional control) and temporal regulation of nutrients and supplements but can also accommodate the growth of follicles by altering the calcium concentration in order to regulate properties of the alginate scaffold. The dynamic customizable system will provide an excellent tool for the *in vitro* culture of human follicles.

In addition, the profuse system will facilitate the monitoring of nutrition uptake and secretion of signalling molecules from the culture. On line monitoring of key biomarkers can be performed in real time. This information will be crucial to understand the biological and molecular process of follicles tissue maturation in vitro.

The success of the *in vitro* culture will be monitored by the number of follicles that survive during a certain period of time, the increase in sizes of the follicles and the morphology of the follicles. We will examine the ability of these follicles to form an antrum (the first sign that a follicle has reached the next stage of maturation) and secrete hormones, so to support oocyte development.

Advances in our understanding of in vitro culturing and maturation of follicles may be applied to provide a fertility preservation option for women at risk of premature ovarian insufficiency as well as facilitating research in folliculogenesis.

4. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Objectives	**Outcome Measures/Endpoints**
Primary Objective To determine a successful in vitro culturing system of early stage human follicles.	• Number of follicles that survive *in vitro* during a certain period of time
Secondary Objectives To identify follicular development and maturation using this system. To further identify factors involved in the in vitro maturation of follicles.	**<u>Secondary endpoint/outcome measures –</u>** Proteomic, metabolomic and genomic studies to determine the success of the *in vitro* culture – measured by: • The increase in size of the follicles • Variations in the morphology of the follicles

	• Measuring numbers of follicles which form an antrum and time taken for antrum to develop • The concentration of hormones secreted by the follicles.

5. STUDY DESIGN

The study design is experimental bench research, using donated human tissue samples.

Patient participation is limited to consenting to the study for the purposes of donating samples only.

In this study donated ovarian tissue will be requested from three patient populations:

1. Female patients having ovarian tissue cryopreservation, from age 6 years onwards, if clinically indicated.
2. Adult women having planned gynaecological surgery:
    - Surgery which already involves the removal of their ovaries (Oophorectomy) or ovarian cysts (Cystectomy). This requires no additional interventions.
    - Surgery to provide sterilisation (i.e. family completed) – surgeons will take a small slice of ovarian tissue (less than 5% total ovary) at the time of surgery from women who agree. This will not affect the endocrine functioning of her ovaries since 10-20% is needed for this.
3. Adult women having elective caesarean section with sterilisation – surgeons will take a small slice of ovarian tissue at the time of surgery from women who agree.

6. PARTICIPANT IDENTIFICATION

6.1. Study Participants

Participants will be female patients who are able to donate ovarian tissue samples to the study.

6.2. Inclusion Criteria

- Participant (or parent/guardian if child unable to consent for themselves) is willing and able to give informed consent for participation in the study.
- Female aged ≥6 and ≤ 43 years having clinically indicated ovarian tissue cryopreservation OR Female aged ≥ 18 and ≤ 43 years and having planned ovarian surgery (Oophorectomy or cystectomy) or sterilisation.

6.3. Exclusion Criteria

- Patients donating new tissue samples (i.e. not samples 'left over' from Oophorectomy or cystectomy) with only one ovary.

7. STUDY PROCEDURES

See **Appendix A** Study Flow Chart for overview of procedures

7.1. Recruitment

All potential study patients (and parent/guardian as appropriate) will have a consultation with a Doctor at a hospital clinic prior to their gynaecological surgery or ovarian tissue preservation surgery. It is at this consultation that the clinic Doctor will present the Patient Information Sheet to suitable patients.

The patient will take the information sheet home and be able to consider the contents. Along with the information sheet we will include a (pre-paid) reply slip which patients will be asked to return if they are interested in taking part in the study.

A research nurse will contact any patient who returns the slip to discuss the study further and, if they wish to take part, will arrange to obtain informed consent at the time when the patients return to the hospital for their surgical procedure.

7.2. Informed Consent

The participant (or parent/guardian if the patient is a child) must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants (or parent/guardian) detailing no less than: the exact nature of the study; what it will

involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant (or parent/guardian) dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant (or parent/guardian). The original signed form will be retained at the study site.

7.3. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason such as Ineligibility (either arising during the study or retrospectively having been overlooked at screening) or withdrawal of consent.

However, in this study patient participation involves the donation of tissue samples only and so their involvement is for a short duration (from consent to removal of tissue). The donated tissue samples are anonymous to the research team (not linked to individuals) and once tissue samples have been given to the researchers they will continue to use the samples even if consent is withdrawn.

7.4. Tissue samples

Surgeons will collect the tissue samples from the study participants and place them in a study labelled container. This will be handed to the research team. The experimental analysis will be carried out immediately in a University laboratory at OCTE. No identifying details are stored.

7.5. Definition of End of Study

The end of the study is the date when the last sample from the last participant has been analysed, and any remaining tissue destroyed.

8. SAFETY REPORTING

We believe it very unlikely our study will be associated with any serious adverse events.

However, the following considerations regarding safety are relevant to this study.

Participants, including children aged 6 and older, are already having planned surgical procedures which require hospitalisation. Study participants are only those patients who are appropriate to donate samples to us and these are:

a) Women already having planned surgery to remove ovarian tissue (whole ovaries in oophorectomy or ovarian cysts in cystectomy). In both these cases the research sample can be taken from the tissue which is already being removed (by the surgeon doing operation) and is therefore 'left over' after the surgery. Nothing extra will happen to these women.

b) Women having planned surgery, including caesarean section, to provide sterilisation (i.e. family completed) – the same surgeon will take a small slice of ovarian tissue (less than 5% total ovary) at the time of surgery from women who agree. This will not affect the endocrine functioning of the ovaries since 10-20% is needed for this.

c) Women and children storing their own ovarian tissue for storage for their own fertility preservation. We will ask this group of patients for their consent to donate a small extra sample for the research study. The surgeon is already doing operation. The extra amount will not affect the procedure they are already having. We are including children is this study as they are most likely to be the population having this procedure and, potentially may be the beneficiaries of this research.

The study interventions will increase their surgery time by 10 minutes only. There are extremely unlikely to be increased surgical risks above that of the planned surgical procedure.

The surgeons are not part of the research team.

Definitions of and reporting of any Serious Adverse Events are described below.

8.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

8.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures.

Reports of related and unexpected SAEs will be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the NRES report of serious adverse event form to the REC/NHS R&D and reported to the sponsor immediately (within 24 hours).

9. STATISTICS AND ANALYSIS

9.1. Description of Statistical Methods

For this study, samples will be analysed using proteomic, metabolomic and genomic methods and the data generated will take the form of experimental measurements to study the factors involved in the in vitro maturation of follicles obtained from human ovarian tissue samples.

9.2. Number of Participants

The number of participants is likely to small but we estimate it to be up to 50 in total.

9.3. Analysis of Endpoints

The samples of ovarian tissue will be collected during the patients' surgery. Samples are put in a labeled container for immediate analysis. No identifying patient data e.g. name, will be retained. Samples are then analysed as follows:

9.3.1. Ovarian follicles isolation

The protocol previously described by Dolmans et al. (Dolmans, Michaux et al. 2006) will be used to

isolate small pre-antral follicles. Briefly, the cortical portion of the ovary was placed in a tissue chopper, adjusted to 0.5 mm. Ovarian fragments obtained are transferred to 50 ml conical flasks containing 10 ml of Dulbecco's phosphate-buffered saline (D-PBS; BioWhittaker, Verviers, Belgium) supplemented with 1 mg/ml collagenase type IA (Sigma), and incubated in a water bath at 37 °C for 60 min with gentle agitation. The ovarian digest is periodically (every 15 min) shaken with a pipette to mechanically disrupt the digested tissue. Digestion is completed by the addition of an equal volume of D-PBS at 4 °C supplemented with 10 % fetal bovine serum (FBS; Gibco). Thereafter, the resulting suspension is centrifuged at 50 g for 10 min at 4 °C. The supernatant is discarded and the pellet is transferred to Petri dishes and investigated for follicles under a stereomicroscope (Leica, Van Hopplynus Instruments, Brussels, Belgium). The follicles are picked up using a 135-mm-diameter stripper tip (Mid-Atlantic Diagnostics, Inc., Mount Laurel, NJ, USA) and washed three times in D-PBS supplemented with 10% FBS at 4 °C in order to avoid introduction of stromal cells into the alginate matrix.

Follicles are then incubated for several days in this media and during this time the follicle diameter is measured from the basement membrane using an ocular micrometer scale. Two to five follicles from each sample are subsequently processed for live/dead assays in order to evaluate follicular viability after isolation. The remaining follicles are embedded in an alginate matrix (4–8 follicles/group).

9.3.2. Follicle and oocyte measurement

To measure the increase in sizes of the follicles

Photographs of each follicle (during the culture period) are captured using a Leica light microscope. Follicle diameters are later measured using ImageJ software (National Institutes of Health, USA). Oocyte diameters are measured without zona pellucida.

Sectioning and staining

To study the morphology of the follicles

Follicles will be fixed in 4% PFA for 30 min at 37 °C and then overnight at 4 °C. Fixed follicles are dehydrated in increasing concentrations of ethanol (10–100%) and embedded in paraffin using an automated tissue processor (Leica, Manheim, Germany). Serial 5 µm sections are cut and stained with hematoxylin and eosin (Xu, Barrett et al. 2009).

9.3.3. 3D confocal staining and imaging

To confirm whether follicles have formed a antrum

Follicles for confocal microscopy studies are fixed in 4 % PFA at 37 °C for 1 hr, followed by 1 hr in wash buffer (Barrett and Albertini 2007). They are then stained overnight at 4 °C on a shaker with Rhodamine-Phalloidin (1:50 Molecular Probes, Invitrogen, Eugene, Oregon) that labels F-actin in TZPs, rabbit anti-connexin 43 (1:100 Zymed) that labels gap junctions or affinity purified polyclonal rabbit antisera that recognize the inhibin βA-subunit (1:100) or the inhibin βB-subunit (1:100). The inhibin subunit antisera is a gift from Dr W. Vale (The Salk Institute, La Jolla, CA, USA). The primary antibodies is followed by incubation with 1:800 goat anti-rabbit Alexa 568 for inhibin βA and connexin 43, 1:800 goat anti-rabbit Alexa 488 for inhibin βB (Molecular Probes) and 1 μg/ml Hoechst 33342 (Molecular Probes) for chromatin/DNA. Follicles are mounted in 5–10 μl of a 50 % glycerol/PBS solution containing 25 μg/ml sodium azide. The coverglass is placed on glass shards, to prevent the compression of the follicle. Follicles are then imaged on a Zeiss LSM 510 confocal microscope using a Neoflaur 40× oil objective. Overlapping 1–3 μm sections are taken throughout each follicle imaged (Xu, Barrett et al. 2009).

9.3.4. Hormone assays

To measure the ability of follicles to secrete hormones

17β-estradiol (E2) and Progesterone (P4) concentrations in culture media were measured by specific electrochemiluminescent assay using a Roche Elecsys 2010 Analyzer (Roche, Indianapolis, IN, USA). Inhibin A, inhibin B and anti-Müllerian hormone (AMH) were measured using ELISA kits (DSL-10-28100, DSL-10-84100 and DSL-10-14400, Diagnostic Systems Laboratories, Webster, TX, USA) following manufacturer instructions (Xu, Barrett et al. 2009).

10. DATA MANAGEMENT

10.1. Access to Data

Direct access will be granted to authorised representatives from the Sponsor or host institution for monitoring and/or audit of the study to ensure compliance with regulations.

10.2. Data Recording and Record Keeping

Copies of consent forms will be kept securely with other study documentation. Samples will be labelled with a Study ID only (anonymous). Data from experiments will be recorded by researchers in laboratory books which are stored securely in a locked building. Data will be transcribed onto Excel spread sheets for analysis. All data is kept on secure University computers and only encrypted data sticks id used. No personal data is available to researchers about the participants.

11. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

12.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

12.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

12.6. Expenses and Benefits

There is no requirement for participants to attend additional visits as part of the study, but if this is the case then we will reimburse reasonable travel expenses on production of receipts, or a mileage allowance provided as appropriate.

13. FINANCE AND INSURANCE

13.1. Funding

Funding is provided by two grants from BBSRC : Engineering human neural network, (BB/H008608), £3,282,711, June 10 – May 15 and Development of a high-throughput perfused three dimensional cell culture platform for stem cell study and drug testing, Taiwan Partnering Award, (BB/L003961/1), £24,991, 1 Sept 13-31 Aug 15.

13.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

14. PUBLICATION POLICY

The investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the BBSRC. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

15. REFERENCES

Abir, R., B. Fisch, et al. (2001). "Morphological study of fully and partially isolated early human follicles." Fertil Steril **75**(1): 141-146.

Abir, R., S. Nitke, et al. (2006). "In vitro maturation of human primordial ovarian follicles: clinical significance, progress in mammals, and methods for growth evaluation." Histol Histopathol **21**(8): 887-898.

Abir, R., P. Roizman, et al. (1999). "Pilot study of isolated early human follicles cultured in collagen gels for 24 hours." Hum Reprod **14**(5): 1299-1301.

Albertini, D. F. and S. L. Barrett (2003). "Oocyte-somatic cell communication." Reprod Suppl **61**: 49-54.

American Cancer Society (2012). Cancer Facts & Figures 2012. . Atlanta.

Amsden, B. and N. Turner (1999). "Diffusion characteristics of calcium alginate gels." Biotechnol Bioeng **65**(5): 605-610.

Andersen, C. Y., M. Rosendahl, et al. (2008). "Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue." Hum Reprod **23**(10): 2266-2272.

Anderson, E. and D. F. Albertini (1976). "Gap junctions between the oocyte and companion follicle cells in the mammalian ovary." J Cell Biol **71**(2): 680-686.

Barrett, S. L. and D. F. Albertini (2007). "Allocation of gamma-tubulin between oocyte cortex and meiotic spindle influences asymmetric cytokinesis in the mouse oocyte." Biol Reprod **76**(6): 949-957.

Borghol, N., J. Lornage, et al. (2006). "Epigenetic status of the H19 locus in human oocytes following in vitro maturation." Genomics **87**(3): 417-426.

Carlsson, I. B., J. E. Scott, et al. (2006). "Anti-Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro." Hum Reprod **21**(9): 2223-2227.

Chang, T. M. (1964). "Semipermeable Microcapsules." Science **146**(3643): 524-525.

Demeestere, I., P. Simon, et al. (2006). "Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report." Hum Reprod **21**(8): 2010-2014.

Demeestere, I., P. Simon, et al. (2007). "Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease." Oncologist **12**(12): 1437-1442.

Dolmans, M. M., N. Michaux, et al. (2006). "Evaluation of Liberase, a purified enzyme blend, for the isolation of human primordial and primary ovarian follicles." Hum Reprod **21**(2): 413-420.

Donnez, J., M. M. Dolmans, et al. (2004). "Livebirth after orthotopic transplantation of cryopreserved ovarian tissue." Lancet **364**(9443): 1405-1410.

Donnez, J., S. Silber, et al. (2011). "Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births." Ann Med **43**(6): 437-450.

Donnez, J., J. Squifflet, et al. (2008). "Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience." Reprod Biomed Online **16**(5): 694-704.

Draget, K. I., G. Skjak-Braek, et al. (1997). "Alginate based new materials." Int J Biol Macromol **21**(1-2): 47-55.

Edwards, R. G. (1965). "Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes." Nature **208**(5008): 349-351.

Galdones, E., L. C. Shea, et al. (2011). Three-dimensional in vitro ovarian follicle culture. Human assisted reproductive technology : future trends in laboratory and clinical practice

D. K. Gardner, B. R. M. B. Rizk and T. Falcone. Cambridge ; New York, Cambridge University Press**:** 167-176.

Gilchrist, R. B., J. E. J. Smitz, et al. (2011). Current status and future trends of the clinical practice of human oocyte in vitro maturation. Human assisted reproductive technology : future trends in laboratory and clinical practice

D. K. Gardner, B. R. M. B. Rizk and T. Falcone. Cambridge ; New York, Cambridge University Press**:** 186-198.

Gremeau, A. S., N. Andreadis, et al. (2012). "In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles." Fertil Steril **98**(2): 355-360.

Heise, M., R. Koepsel, et al. (2005). "Calcium alginate microencapsulation of ovarian follicles impacts FSH delivery and follicle morphology." Reprod Biol Endocrinol **3**: 47.

Hovatta, O., R. Silye, et al. (1997). "Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture." Hum Reprod **12**(5): 1032-1036.

Hovatta, O., C. Wright, et al. (1999). "Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation." Hum Reprod **14**(10): 2519-2524.

Kim, B. S. and D. J. Mooney (1998). "Development of biocompatible synthetic extracellular matrices for tissue engineering." Trends Biotechnol **16**(5): 224-230.

Kreeger, P. K., J. W. Deck, et al. (2006). "The in vitro regulation of ovarian follicle development using alginate-extracellular matrix gels." Biomaterials **27**(5): 714-723.

Kreeger, P. K., N. N. Fernandes, et al. (2005). "Regulation of mouse follicle development by follicle-stimulating hormone in a three-dimensional in vitro culture system is dependent on follicle stage and dose." Biol Reprod **73**(5): 942-950.

Li, Y., H. L. Feng, et al. (2006). "Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro." Fertil Steril **85**(4): 827-832.

Meirow, D., J. Levron, et al. (2007). "Monitoring the ovaries after autotransplantation of cryopreserved ovarian tissue: endocrine studies, in vitro fertilization cycles, and live birth." Fertil Steril **87**(2): 418 e417-418 e415.

Meirow, D., J. Levron, et al. (2005). "Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy." N Engl J Med **353**(3): 318-321.

Monniaux, D., C. Huet-Calderwood, et al. (2006). "Integrins in the ovary." Semin Reprod Med **24**(4): 251-261.

Murray, A. A., R. G. Gosden, et al. (1998). "Effect of androgens on the development of mouse follicles growing in vitro." J Reprod Fertil **113**(1): 27-33.

Nebel, R. L., J. H. Bame, et al. (1985). "Microencapsulation of bovine spermatozoa." J Anim Sci **60**(6): 1631-1639.

Oktay, K., E. Buyuk, et al. (2004). "Embryo development after heterotopic transplantation of cryopreserved ovarian tissue." Lancet **363**(9412): 837-840.

Oktay, K., H. Newton, et al. (2000). "Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice." Fertil Steril **73**(3): 599-603.

Oktay, K., D. Nugent, et al. (1997). "Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue." Fertil Steril **67**(3): 481-486.

Pangas, S. A., H. Saudye, et al. (2003). "Novel approach for the three-dimensional culture of granulosa cell-oocyte complexes." Tissue Eng **9**(5): 1013-1021.

Radford, J. A., B. A. Lieberman, et al. (2001). "Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma." Lancet **357**(9263): 1172-1175.

Reynaud, K., R. Cortvrindt, et al. (2000). "Effects of Kit Ligand and anti-Kit antibody on growth of cultured mouse preantral follicles." Mol Reprod Dev **56**(4): 483-494.

Rosendahl, M., A. Loft, et al. (2006). "Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report." Hum Reprod **21**(8): 2006-2009.

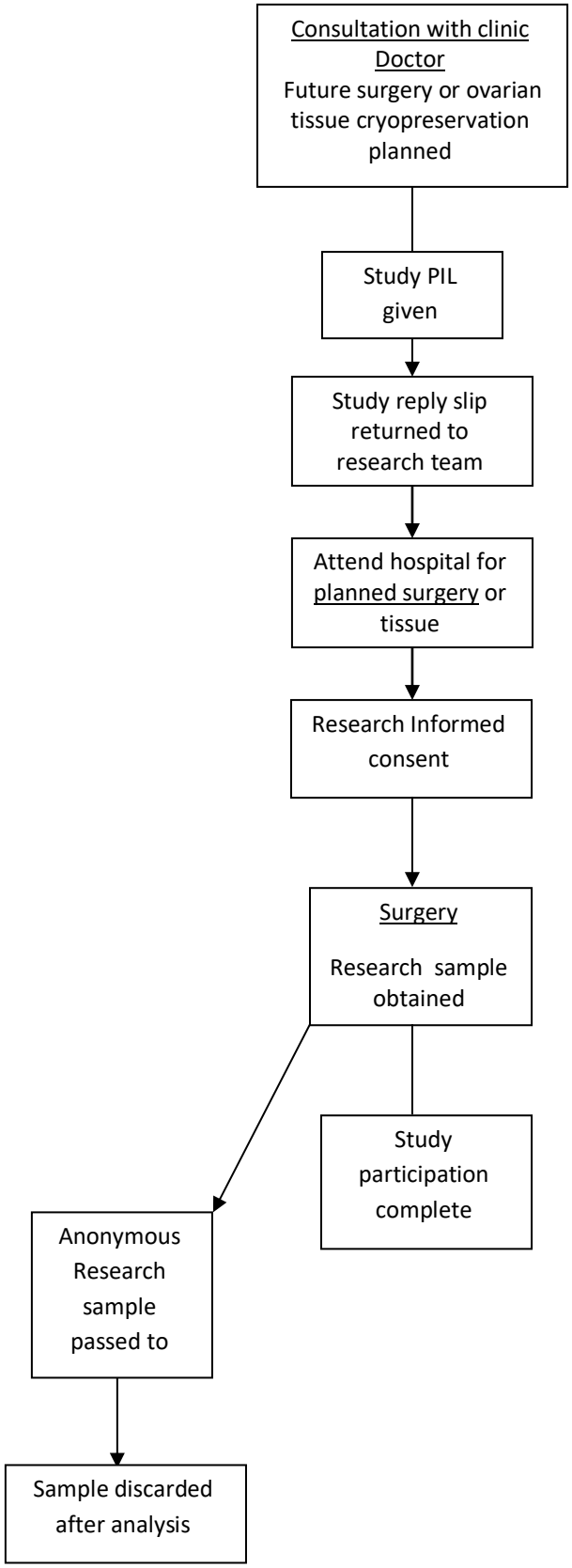
Roy, S. K. and B. J. Treacy (1993). "Isolation and long-term culture of human preantral follicles." Fertil Steril **59**(4): 783-790.

Schmidt, K. L., C. Y. Andersen, et al. (2005). "Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation." Hum Reprod **20**(12): 3539-3546.

Smidsrod, O. and G. Skjak-Braek (1990). "Alginate as immobilization matrix for cells." Trends Biotechnol **8**(3): 71-78.

Smitz, J. and R. Cortvrindt (1998). "Follicle culture after ovarian cryostorage." Maturitas **30**(2): 171-179.
Tan, S. L. and T. J. Child (2002). "In-vitro maturation of oocytes from unstimulated polycystic ovaries." Reprod Biomed Online **4 Suppl 1**: 18-23.
Telfer, E. E., M. McLaughlin, et al. (2008). "A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin." Hum Reprod **23**(5): 1151-1158.
Torre, M. L., M. Faustini, et al. (2007). "Cell encapsulation in mammal reproduction." Recent Pat Drug Deliv Formul **1**(1): 81-85.
Tryde Schmidt, K. L., C. Yding Andersen, et al. (2004). "Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer - follicular growth, steroid production and oocyte retrieval." Reprod Biomed Online **8**(4): 448-453.
West, E. R., M. Xu, et al. (2007). "Physical properties of alginate hydrogels and their effects on in vitro follicle development." Biomaterials **28**(30): 4439-4448.
Xu, M., S. L. Barrett, et al. (2009). "In vitro grown human ovarian follicles from cancer patients support oocyte growth." Hum Reprod **24**(10): 2531-2540.
Xu, M., P. K. Kreeger, et al. (2006). "Tissue-engineered follicles produce live, fertile offspring." Tissue Eng **12**(10): 2739-2746.
Xu, M., E. West, et al. (2006). "Identification of a stage-specific permissive in vitro culture environment for follicle growth and oocyte development." Biol Reprod **75**(6): 916-923.

16. APPENDIX A: STUDY FLOW CHART



17. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

Study Title: **In Vitro Culture and Maturation of Ovarian Tissue and Follicles: Follicle microenvironment engineering as a tool to study follicle development and provide fertility preservation.**

Short title: Methods of ovarian tissue culturing for fertility preservation

Ethics Ref 14/SC/0041

Date and Version No: V2.0 27 January 2016

Chief Investigator:	Dr. Suzannah Williams, Senior Research Fellow, University of Oxford, Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford, OX3 9DU. Tel 01865-221014
Investigators:	Muhammad Fatum, Clinical Research Fellow, University of Oxford, Nuffield Department of Obstetrics & Gynaecology, Institute of Reproductive Sciences, Oxford Business Park North, Oxford, OX4 2HW. Tel 01865-782-876
	Prof Z. F. Cui, Director, Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, Parks Road OX1 3PJ
Sponsor:	University of Oxford
Funder :	BBSRC Grant (-2015), Discretionary account at the Department of Obstetrics and Gynaecology
Signature:	The approved protocol should be signed by author(s) and/or person(s) authorised to sign the protocol

There are no conflicts of interest declared.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

TABLE OF CONTENTS

1. SYNOPSIS

Study Title	**In Vitro Culture and Maturation of Ovarian Tissue and Follicles: Follicle Microenvironment Engineering as a tool to study follicle development and provide fertility preservation**	
Short title	Methods of ovarian tissue culturing for fertility preservation	
Study Design	Bench research using donated human tissue samples	
Study Participants	Females aged 6-43 having clinically indicated ovarian tissue cryopreservation Women aged 18-43 having planned ovarian surgery or sterilisation	
Planned Sample Size	Up to 50	
Planned Study Period	5 years	
	Objectives	**Endpoints**
Primary	To determine a successful *in vitro* culturing system of early stage human follicles.	Number of follicles that survive *in vitro* during a certain period of time
Secondary	To analyse follicular development and maturation using this system. To further identify factors involved in the in vitro maturation of follicles.	Proteomic, metabolomic and genomic studies to determine the success of the *in vitro* culture – measured by: • The increase in size of the follicles • Variations in the morphology of the follicles • Measuring numbers of follicles which form an antrum and time taken for antrum to develop • The concentration of hormones secreted by the follicles.

2. ABBREVIATIONS

CI	Chief Investigator

CRF	Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
GCP	Good Clinical Practice
GP	General Practitioner
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IVF	In Vitro Fertilisation
IVM	In Vitro Maturation
NHS	National Health Service
NRES	National Research Ethics Service
OCTE	Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RGD	Three dimensional (3D) scaffold and adhesion motif
SOP	Standard Operating Procedure

3. BACKGROUND AND RATIONALE

3.1. Overview

For female adolescents and young adults, the increasing probability of surviving a cancer diagnosis combined with the expectation and desire for reproductive options following cancer remission has fuelled a growing need for fertility sparing techniques.

Currently there are several methods available to women hoping to preserve their fertility before cancer therapy. As it has been used most often, traditional hormone stimulation and in vitro fertilization (IVF) followed by embryo cryopreservation is the most successful approach to preserve fertility. More recently live births have also been achieved with cryopreserved oocytes harvested before cancer treatment. Both methods require a delay in cancer treatment and use hormonal stimulation that might be deleterious in some patients. Ovarian tissue cryopreservation followed by autotransplantion is a promising fertility preservation approach that can usually be performed immediately without hormonal stimulation.

Worldwide, a dozen live births have been reported thus far as a result of auto-transplanting frozen/thawed ovarian tissues (Donnez, Silber et al. 2011). Despite these promising findings, transplantation of cryopreserved tissue carries the risk of re-introducing cancer cells into the patient. Thus, the in vitro maturation (IVM) of immature follicles derived from ovaries collected prior to commencement of cancer treatment is an important direction for new fertility preservation approach as it avoids the need for auto grafting and prevents the possibility of cancer cells reseeding.

In this proposal we aim to establish follicle culture using tissue from human ovarian biopsies and also improve them by exploring the use of three dimensional (3D) scaffold and adhesion motif (RGD). These 3D culture systems can potentially improve follicle growth and development via enhances structural support. Furthermore, maintaining the follicular structure while monitoring and manipulating the hormonal and biochemical environment through the perfusion system will allow us to explore the development of controllable systems to investigate and improve the biological and molecular processes underlying follicle growth and maturation. Advances in our understanding of *in vitro* culturing and maturation of follicles may also be applied to provide a fertility preservation option for women at risk of premature ovarian insufficiency as well as facilitating research in follicle development.

3.2. Fertility Preservation in Cancer Patients

In recent years cryopreservation of human ovarian tissues has emerged as a promising alternative for fertility preservation in pre-menarchal and adolescent females, as well as for adult women prior to chemo/radiotherapy. The prognosis and survival rates of cancer patients have improved tremendously as a result of recent advances in cancer treatment, particularly in childhood cancers, and attention is now being directed towards quality of life and the long-term gonadotoxic side effects of chemotherapy or radiotherapy. Given that 1 in 46 females between birth and age 39 are diagnosed with cancer(American Cancer Society 2012) many women are potentially affected. The incidence of ovarian failure is dependent on the type and dose of chemotherapy agents, and the age of the patient. Currently, there are several modalities for fertility preservation for different age groups:

Post-pubertal women

Post pubertal women requesting fertility preservation prior to chemotherapy or radiotherapy may be offered any of the following options:

3.2.1. Embryo cryopreservation

This is currently the only widely accepted and well-established option to preserve fertility, during which patients undergo a stimulated IVF cycle with subsequent retrieval of mature oocytes prior to the initiation of chemo/radiotherapy. Harvested oocytes are then fertilized with sperm produced by husband, partner or donor, and the resulting embryos are cryopreserved. This technology is in routine clinical use in IVF units throughout the world on a daily basis. An evolving newer strategy is obtaining immature oocytes in non-stimulated cycle for in vitro maturation of oocytes and subsequent fertilization, and embryo freezing.

The advantages of embryo cryopreservation modality are its worldwide availability and its proven and established pregnancy success rates. Disadvantages include the need for hormonal stimulation of 2-4 weeks (which may defer and delay the anticancer treatment), its costs (£4000-5000), the need for a partner/husband or the decision of using donor sperm, and the relatively limited number of embryos produced and stored from a single stimulation cycle. It is not a viable option for pre-menarchal or adolescent girls, single women unable/unwilling to use donor sperm and women who are unable to undergo an *in vitro* fertilization cycle due to the need to undergo immediate anticancer treatment. In addition, the IVF procedure, especially hormonal stimulation, causes discomfort and increases the risk for OHSS, ovarian, breast and endometrium cancer, and stroke (Gilchrist et al., 2011). Additionally, there are ethical issues regarding embryo storage and usage.

3.2.2. Oocyte cryopreservation

In this modality, the patient undergoes a stimulated IVF cycle and subsequently the harvested oocytes are cryopreserved for later thawing out and fertilization. This technology can be an option for single female patients who are not in a stable relationship and not willing to opt for sperm donation. Because of the large size and more complex and fragile nature of the oocyte, oocyte freezing has been technically difficult with limited proven success rates. The introduction of rapid vitrification is promising, as it has been shown to have much elevated oocyte survival rates. However, the issues with the hormonal stimulation described above remain.

A newer strategy of oocyte preserving involves obtaining immature oocytes from unstimulated ovaries. These immature oocytes, which are smaller-sized, less complicated structurally and metabolically less active than mature oocytes, are either subsequently frozen or cultured in an *in vitro* system and cryopreserved as mature oocytes. The merits of this option include the sparing of the ovulation induction process; as a result, it can be performed immediately, avoiding the delay of chemo/radiotherapy and has

lower risks related to exposure of high oestradiol levels. In addition, it requires less patient monitoring and reduces the associated costs. The challenge is the *in vitro* maturation process.

3.2.3. Ovarian tissue cryopreservation

In this approach an operative procedure (usually laparoscopy, rarely laparotomy) is performed, prior to commencement of anticancer treatment, to harvest ovarian tissue. This is cryopreserved and is followed by auto-transplantation later in life i.e. following recovery from the cancer treatment and when patient is ready to proceed with fertility treatment. The so called autografting of the cryopreserved tissue involves transplanting the ovarian tissue back into the patient immediately after thawing, either to the ovarian fossa (orthotopic graft) or to a different site (heterotopic graft). With autografting, immature follicles and oocytes would mature in *vivo* (thus obviating the need for exogenous gonadotropin stimulation) or ovulation induction treatment or IVF cycle may be needed. This treatment is still considered experimental, albeit a promising one for fertility preservation. An increasing number of pregnancies are being reported and thus far approximately 13 live births have been reported using this technology (Donnez, Silber et al. 2011).

As with immature oocyte cryopreservation, this approach can be performed immediately, often without hormonal stimulation. It may be offered to women undergoing aggressive chemotherapy/radiotherapy with a high likelihood of gonadotoxicity. This modality may be suggested as well for fertility preservation in young women to allow future childbearing through assisted conception without the risk of age related loss of fertility, the so called- social indication. There are several points to be taken into account when considering this approach and to tailor treatment to the individual concerned: the reproductive age, the chance of survival and the relative risk of subfertility related to the chemotherapy versus the possibility of the re-introduction of cancer cells and other clinical considerations.

3.2.4. Other options for fertility preservation

Ovarian suppression prior to chemotherapy

The use of GnRH analogues for ovarian suppression is controversial, and with no hitherto established or well-proven value. Its use should be considered in well-designed experimental protocols.

Oophoropexy prior to radiotherapy:

This modality includes an operative procedure, mainly laparoscopy, to move ovaries out of the pelvic radiotherapy field. It would be appropriate for only a very few well-defined patients.

Pre-pubertal children:

Ovarian tissue cryopreservation, though still considered as an experimental approach, is the only available fertility preservation approach that can be offered for premenarcheal/ pre-pubertal girls. Currently, this technique is available in only a limited number of fertility centres in several countries. It has been used in children as young as 2.7 years of age.

In summary, ovarian tissue preservation is a feasible option to preserve ovarian function and possibly fertility in children and young women before gonadotoxic chemotherapy and/or radiotherapy. However, further research is needed to assess the clinical effectiveness of ovarian cryopreservation, to optimise the technique and to define clear indications.

3.3. Challenges with Ovarian Tissue Preservation

Restoration of ovarian function with hormone production and follicular growth has been observed after transplantation of frozen–thawed ovarian tissue (Oktay, Newton et al. 2000, Radford, Lieberman et al. 2001, Oktay, Buyuk et al. 2004, Tryde Schmidt, Yding Andersen et al. 2004, Schmidt, Andersen et al. 2005, Rosendahl, Loft et al. 2006, Donnez, Squifflet et al. 2008), and more than a dozen of pregnancies (Donnez, Dolmans et al. 2004, Meirow, Levron et al. 2005, Demeestere, Simon et al. 2006, Demeestere, Simon et al. 2007, Meirow, Levron et al. 2007, Andersen, Rosendahl et al. 2008) have been reported to date.

Nevertheless research efforts are still needed to develop and optimize grafting procedures; a large percentage of grafted follicles are lost as a result of post grafting ischemia and reperfusion-induced damage (Newton et al., 1996; Aubard et al., 1999; Donnez et al., 2006b). In addition, some studies have demonstrated the risk of possibly transmitting malignant cells present in the cryopreserved tissue back to patient's body (Shaw et al., 1996; Meirow et al., 1998, 2008). Thus, an important direction for research is into ways to avoid the need for autografting, such as the *in vitro* development of immature follicles derived from ovaries collected prior to commencement of cancer treatment. A successful *in vitro* culture system for ovarian tissue would then enable the growth and maturation of follicles and oocytes which could be subsequently fertilized to produce viable embryos.

3.4. In vitro culture and maturation of follicles

In order to avoid the risk of transmitting cancer cells as well as other technical challenges of grafting including long term biocompatibility, matrix degradation vascularization, and integration with existing

tissue (Kim and Mooney 1998, West, Xu et al. 2007), isolated follicles could be grown *in vitro* and then oocytes isolated for *in vitro* maturation (IVM) followed by fertilization and embryo transfer. The follicle culture procedure would also allow the assessment of follicle quality after cryopreservation by direct monitoring of follicles during the culture period (Abir, Roizman et al. 1999), which could also help to assess the factors involved in the follicle development in humans.

The first human oocyte IVM was performed by Edwards in 1965 (Edwards 1965). The purpose of *in vitro* human follicle growth is to mimic the *in vivo* process by providing follicles with appropriate growth factors and hormones, in the correct amount and at the right time, to enable growth of the follicle and oocyte whilst maintaining the essential connections between somatic cells and the oocyte (called transzonal projections (TZPs)) (Anderson and Albertini 1976, Albertini and Barrett 2003, Xu, Barrett et al. 2009). At the moment IVM is regarded as an adjunct procedure to IVF but successful *in vitro* culturing and maturation of the ovarian tissue or isolated follicles to produce mature follicles and oocytes, would provide a safe fertility preservation option.

Currently there are two standard clinical protocols for IVM (Gilchrist, Smitz et al. 2011). First, the cumulus-oocyte complex is removed when the woman's endometrium thickness is at least 5 mm. The complex removed from the meiotically inhibiting environment is spontaneously matured to the metaphase II stage in 36 hours. The other method involves a large bolus of Human Chorionic Gonadotrophin (HCG) administration, to initiate meiotic resumption, 36 hours prior to oocyte retrieval. In this case IVM time is significantly reduced to 24 hours. The *in vitro* culture systems can also be categorized into two approaches. In the first approach isolated follicles are placed on two-dimensional surface and permitted to attach and spread on the surface. The second one is the three dimensional intact follicle approach in which follicles do not adhere to a surface but maintain their architecture and the cell-cell and cell-matrix interactions which are critical regulators of follicle development (West et al., 2007).

These current clinical IVM methods only support late stage pre-ovulatory follicles. However, as the procedures depend on the hormonal regulation and the availability of antral follicles, they are not yet an option for pre-pubertal children and cancer patients who require urgent treatment. The ability to culture immature follicles will be invaluable for fertility preserving technologies. Primordial follicles are the most abundant follicle stage in the ovary and present at all ages. They are also less susceptible to cryogenic and cancer therapy damage.

Therefore, culture techniques for pre-antral follicles should be developed, although to date the culture of early stage (primordial or primary) follicles to the antral stage has yet to be fully elucidated (Galdones,

Shea et al. 2011) and only a few studies have been performed and published (Roy and Treacy 1993, Oktay, Nugent et al. 1997, Abir, Roizman et al. 1999, Abir, Fisch et al. 2001). However, although the *in vitro* culture of isolated small pre-antral follicles (30–50 μm) from human ovaries has not yet yielded satisfactory results, samples from fresh and frozen–thawed ovarian tissue have been shown to survive *in vitro* for up to 24 hours (Abir, Roizman et al. 1999, Abir, Fisch et al. 2001) in collagen gel.

At the moment, two step approaches seem to be promising in the development of a method for the culture of primary follicles. Because of the different requirements of the primary and secondary follicles, two separate systems were developed to support each development stage. First, primary follicles in *ex vivo* ovarian tissue were cultured to secondary follicles. Then the secondary follicles were collected and cultured in an encapsulated system. Murine fetal ovaries and cryopreserved ovaries have been cultured successfully using this two-step method.

Unfortunately, another challenge of *in vitro* human follicle culture is that most follicle culture studies have previously been done in rodents where the terminal follicle size is smaller and the culture period is shorter. The increased volume means the follicles are more susceptible to sheer and diffusion limitation. The human ovary also contains more fibrous stroma which makes follicle collection difficult. Telfer and colleagues tried to culture human preantral follicles using the two step culture system, but the follicles could maintain their structure only up to 4 days (Telfer, McLaughlin et al. 2008). However, human secondary follicles could be cultured to the antral stage in the presence of activin.

Animal studies have shown that the major problem in early stage follicle culture is how to maintain the oocyte development competence in the artificial environment. Despite all attempts to manipulate the culture conditions, the differences between cellular environment in standard 2D cell culture system and the real physiological environment usually exist and are significant, which results in the deviated cellular behaviour. For example, more abnormal chromosome configurations and disturbed methylation pattern on the gene H19 are observed in IVM oocyte compared to oocyte matured *in vivo* under ovarian stimulation (Borghol, Lornage et al. 2006, Li, Feng et al. 2006). As a result, these oocytes are likely to fail to mature and result in high miscarriage rate of IVM derived embryos.

Most IVM researchers have tried to improve IVM conditions by optimizing culture medium formula. Pre-IVM treatment with cyclic adenosine monophosphate (cAMP) modulating agents and addition of, for example, FF-MAS, oocyte secreting factors, protein kinase c activator, have shown to enhance the embryo yield and the fetal survival significantly see Gilchrist for reviews (Gilchrist, Smitz et al. 2011). Nevertheless, Abir et al. reported that isolated follicles could only grow in a supporting matrix i.e. a 3D system (Abir, Nitke et al. 2006), possibly due to the preservation of intercellular interactions between

granulosa cells (GCs) and the oocyte in this system and the provision of optimal support for the fragile isolated follicles, similar to the ovary itself.

3.4.1. 3D scaffold

In the ovary, primordial and primary follicles are localized to the rigid part of the ovary and migrate to the less rigid part while growing. The presence of 3D scaffold and its rigidity are sensed through cellular contact that subsequently triggers various signalling cascades for cell survival and differentiation. Subsequently, cells survival period and tissue integrity are enhanced.

Two-dimensional cultures have been revealed as insufficient for sophisticated cell culture. Over time the structure of the spherical follicle is disrupted as it flattens and the loss of follicular structure releases denuded oocytes. The degree of disruption increases with the terminal size of the follicles. 2D follicle tissue culture also leads to granulosa cell proliferation, migration from the oocyte, and subsequent flattening of the oocyte (Pangas, Saudye et al. 2003).

Morphophysiological observations suggest that the suitable conditions for *in vitro* oocyte maturation maintain its cellular environment and well defined extracellular matrix structure in 3D organization (Torre, Faustini et al. 2007). Many aspects of oocyte growth and development are believed to be regulated by interactions with adjacent GCs (Murray, Gosden et al. 1998, Reynaud, Cortvrindt et al. 2000) and this is probably why most studies are performed with pre-antral follicles enclosed in ovarian tissue (Hovatta, Silye et al. 1997, Hovatta, Wright et al. 1999, Carlsson, Scott et al. 2006).

Cell encapsulation is the technique whereby a pool of live cells is entrapped within a semi permeable membrane. Since the first publication by Chang et al in 1964 (Chang 1964), bioencapsulation has gained interest from researchers. The first application of cell encapsulation in reproduction was made by Nebel et al. who devised a technique for bovine sperm encapsulation in calcium alginate and polyamines (Nebel, Bame et al. 1985). Later, Pangas et al. developed an alginate beads 3D culture system designed for cumulus/oocyte complexes (Pangas, Saudye et al. 2003); such technology yielded good results in terms of structural development and meiosis resumption for murine ovary. 3D culture scaffold will slow the structural deterioration of the spherical follicle by giving mechanical support (Smitz and Cortvrindt 1998).

3.4.2. Alginate Hydrogel

Bioactive material such as collagen and Matrigel would appear to be excellent scaffold for *in vitro* follicle

culture however, since the matrices are bioactive, some degree of cell migration from the follicle and enzyme activity can lead to changes in the follicle structure and follicular damage. Inert 3D matrix such as alginate or polyethylene glycol (PEG) has proven to be a well characterized and customizable tool for *in vitro* culture.

Alginate is a nontoxic polysaccharide composed of repeating units of mannuronic (M) and glucuronic acids (G) that may be reversibly linked by exposure to divalent cations such as calcium (Ca^{2+})(Pangas, Saudye et al. 2003, Torre, Faustini et al. 2007). It is permeable to small molecules such as glucose and oxygen and its permeability can be easily tailored to suit different applications. The alginate encapsulation procedure does not damage the ovarian cells or cellular junctions. It results in a surrounding alginate matrix that maintains the positions of the granulosa cells within the oocyte; hence preserving the granulosa cell–oocyte interactions in the presence of appropriate growth and differentiation factors (Pangas, Saudye et al. 2003). Also, oocytes grown *in vitro* can be readily retrieved from the matrix for further uses by the addition of a chelating agent such as EDTA or sodium citrate. Further uses could include *in vitro* fertilisation. According to the properties described above, alginate hydrogel should be suitable for *in vitro* culture of isolated follicles. Its porous structure allows diffusion of hormones and other proteins that are essential for follicular development. Indeed, alginate is one of the most widely used biomaterials for microencapsulation because of its biocompatibility, high affinity to water and ability to form hydrogels under very mild conditions (Smidsrod and Skjak-Braek 1990, Draget, Skjak-Braek et al. 1997, Amsden and Turner 1999, West, Xu et al. 2007).

Moreover, alginate scaffold can be functionalised for specific use. Small proteins and functional groups can be attached to the alginate scaffold by chemical modification. The rigidity and mechanical properties of the alginate can be altered by the ratio of G:M monomer and calcium concentration in the linking solution. The dynamic characteristics of alginate scaffold make it a suitable material for robust dynamic *in vitro* follicle culture.

A fluid core alginate capsule can support human oocyte maturation well with 90.3% success rate after 48 hr. Recently, another 3D alginate hydrogels system has been successfully applied to in vitro culture of isolated mouse (Pangas, Saudye et al. 2003, Kreeger, Fernandes et al. 2005, Kreeger, Deck et al. 2006, Xu, Kreeger et al. 2006, Xu, West et al. 2006, West, Xu et al. 2007) and rat (Heise, Koepsel et al. 2005) follicles. Mouse follicle encapsulated in alginate beads culture in 100 uL growth medium (a-MEM-0.3% BSA, fetuin 1 mg/mL) for 10 days grew as intact spheres of cells within the alginate bead. At this stage, the oocytes showed no signs of degeneration, had cortical granules around the periphery, and contained an intact zona pellucida with numerous microvilli projecting from the oocyte. The granulosa cells surrounding the oocyte were organized, spherical and had few vacuoles. No morphological evidence of

cellular apoptosis was noted (Pangas, Saudye et al. 2003). Follicles cultured in alginate produced fertilizable oocytes and live mice have been born from these (Pangas, Saudye et al. 2003, Xu, Kreeger et al. 2006). However, alginate exhibits minimal cellular interactions with mammalian cells, and thus likely provides only mechanical support.

3.4.3. Adhesion motif RGD

Along with the signalling molecules between cells in the follicle, the interaction between follicle cells and the surrounding extracellular matrix (ECM) also appear to contribute to follicle fate (Irving-Rodges et al, 2009). Most of the inert scaffolds lack interaction with the cells. They are coated with cell surface adhesive proteins such as collagen, laminin and fibronectin to enhance cell attachment. However, these proteins are usually derived from animals so they may be contaminated with pathogen or trigger the immune response. Also, since they are susceptible to denaturation and degradation, they need to be replenished constantly. What is more the binding of large protein molecules onto scaffold backbone molecules such as alginate is not easy technically. To overcome these drawbacks, immobilized peptides are presented as the cell recognition motifs instead. ECM usually contains several protein motives to interact with specific receptors. The smaller peptide motif is more stable and can be packed at higher density.

RGD peptide is a cell recognition motif that consists of Arginine (R), Glycine (G), Aspartic acid (D). It is recognized by cell surface receptors of the integrin family. Integrins are the major receptors mediating adhesion to the extracellular matrix. Following ligand binding, conformational changes of integrins induce the recruitment of multiple signalling and scaffolding proteins that connect integrin tails to the actin cytoskeleton and permit activation of signalling pathways regulating cell proliferation, apoptosis, differentiation, and migration. In the ovary, extracellular matrix components present in the follicular basement membrane, around follicular cells, and in the follicular fluid and the role of integrins in the regulation of follicular development is strongly suggested (Monniaux, Huet-Calderwood et al. 2006). Smitz et al. reported that the presence of an extracellular matrix enhances follicle survival, especially after 10 days in mouse follicle culture and 14 days in sheep follicle culture (Smitz and Cortvrindt 1998), but there is no significant difference in follicle diameters with ECM culture compared to non-ECM culture.

Kreeger et al showed that encapsulation and culture of follicles with RGD-modified alginate significantly increased growth of secondary follicles and improved meiotic competency rates of oocytes compared with follicles cultured in alginate alone (Kreeger, Deck et al. 2006).

3.4.4. Perfused system

Follicles at different stages require different culture conditions. Consequently, well defined and well controlled culture environment will be the key to the success of *in vitro* follicle culture and this can only be achieved in a dynamic perfused culture system. In the conventional static culture environment all parameters in the culture medium change with time depending on metabolic rate and local conditions. Perfused technology, where culture media is pumped and flows through the culture at a pre-set manner, not only allows the better transportation (i.e. overcome diffusional control) and temporal regulation of nutrients and supplements but can also accommodate the growth of follicles by altering the calcium concentration in order to regulate properties of the alginate scaffold. The dynamic customizable system will provide an excellent tool for the *in vitro* culture of human follicles.

In addition, the perfusion system will facilitate the monitoring of nutrition uptake and secretion of signalling molecules from the culture. On line monitoring of key biomarkers can be performed in real time. This information will be crucial to understand the biological and molecular process of follicles tissue maturation in vitro.

The success of the *in vitro* culture will be monitored by the number of follicles that survive during a certain period of time, the increase in sizes of the follicles and the morphology of the follicles. We will examine the ability of these follicles to form an antrum (the first sign that a follicle has reached the next stage of maturation) and secrete hormones, so to support oocyte development.

Advances in our understanding of in vitro culturing and maturation of follicles may be applied to provide a fertility preservation option for women at risk of premature ovarian insufficiency as well as facilitating research in follicle development.

3.4.5. Reaggregated ovary culture

Follicle development requires complex coordinated interactions between the oocyte and somatic cells with the oocyte secreting factors that influence its surrounding cells. In around half of women with premature ovarian failure (POF), the ovary still contains primordial follicles but ovarian function has ceases preventing their development; this is known as follicular POF. These cells can be obtained and combined with an alternate source of somatic cells that can support oocyte development; these are known as a reaggregated ovary (RO). ROs can be grown *in vivo* by transplanting the RO under the kidney capsule of a host mouse for 3 weeks where follicles at all stages of development can be generated (Eppig and Wigglesworth, 2000). The critical feature is that ROs can be generated using germ cells from one source and somatic cells from another.

Recently, the Williams Lab has established an *in vitro* RO culture technique using mouse germ and somatic cells which enables follicles to develop to the large antral stage. The aim of adapting this technology to grow human germ cells *in vitro* within an RO is to develop a treatment for follicular POF and also for women with ovarian cancer.

ROs can be developed *in vivo* using mouse, rat and bovine cells and studies have demonstrated that somatic cells from one species can support oocyte development in another: rat and mouse (Eppig and Wigglesworth, 2000). Therefore, since we wish to adapt this technology to develop human eggs *in vitro*, initial experiments will include the use of somatic cells from alternate sources to support human oocyte development in an RO. One example of an alternate somatic cell are mouse somatic cells because we understand their function *in vitro*, we have demonstrated that they support oocyte development of mouse oocytes *in vivo* and *in vitro* and bovine oocytes *in vivo*, and therefore these will be a useful tool in our aim of establishing reliable human oocyte development *in vitro*.

4. OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

Objectives	**Outcome Measures/Endpoints**
Primary Objective To determine a successful in vitro culturing system of early stage human follicles.	• Number of follicles that survive *in vitro* during a certain period of time
Secondary Objectives To analyse follicular development and maturation using this system. To further identify factors involved in the in vitro maturation of follicles.	**<u>Secondary endpoint/outcome measures –</u>** Proteomic, metabolomic and genomic studies to determine the success of the *in vitro* culture – measured by: • The increase in size of the follicles • Variations in the morphology of the follicles • Measuring numbers of follicles which form an antrum and time taken for antrum to develop • The concentration of hormones secreted by the follicles.

5. STUDY DESIGN

The study design is experimental bench research, using donated human tissue samples.

Patient participation is limited to consenting to the study for the purposes of donating samples only.

In this study donated ovarian tissue will be requested from three patient populations:

1. Female patients having ovarian tissue cryopreservation, from age 6 years onwards, if clinically indicated.
2. Adult women having planned gynaecological surgery:
    - Surgery which already involves the removal of their ovaries (Oophorectomy) or ovarian cysts (Cystectomy). This requires no additional interventions.
    - Surgery to provide sterilisation (i.e. family completed) – surgeons will take a small slice of ovarian tissue (less than 5% total ovary) at the time of surgery from women who agree. This will not affect the endocrine functioning of her ovaries since 10-20% is needed for this.
3. Adult women having elective caesarean section with sterilisation – surgeons will take a small slice of ovarian tissue at the time of surgery from women who agree.

6. PARTICIPANT IDENTIFICATION

6.1. Study Participants

Participants will be female patients who are able to donate ovarian tissue samples to the study.

6.2. Inclusion Criteria

- Participant (or parent/guardian if child unable to consent for themselves) is willing and able to give informed consent for participation in the study.
- Female aged ≥6 and ≤ 43 years having clinically indicated ovarian tissue cryopreservation OR Female aged ≥ 18 and ≤ 43 years and having planned ovarian surgery (Oophorectomy or cystectomy) or sterilisation.

6.3. Exclusion Criteria

- Patients donating new tissue samples (i.e. not samples 'left over' from Oophorectomy or cystectomy) with only one ovary.

7. STUDY PROCEDURES

See **Appendix A** Study Flow Chart for overview of procedures

7.1. Recruitment

All potential study patients (and parent/guardian as appropriate) will have a consultation with a Doctor at a hospital clinic prior to their gynaecological surgery or ovarian tissue preservation surgery. It is at this consultation that the clinic Doctor will present the Patient Information Sheet to suitable patients.

The patient will take the information sheet home and be able to consider the contents. Along with the information sheet we will include a (pre-paid) reply slip which patients will be asked to return if they are interested in taking part in the study.

A research nurse will contact any patient who returns the slip to discuss the study further and, if they wish to take part, will arrange to obtain informed consent at the time when the patients return to the hospital for their surgical procedure.

7.2. Informed Consent

The participant (or parent/guardian if the patient is a child) must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants (or parent/guardian) detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant (or parent/guardian) dated signature and dated signature of the person who presented and obtained the

Informed Consent. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant (or parent/guardian). The original signed form will be retained at the study site.

7.3. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason such as Ineligibility (either arising during the study or retrospectively having been overlooked at screening) or withdrawal of consent.

However, in this study patient participation involves the donation of tissue samples only and so their involvement is for a short duration (from consent to removal of tissue). The donated tissue samples are anonymous to the research team (not linked to individuals) and once tissue samples have been given to the researchers they will continue to use the samples even if consent is withdrawn.

7.4. Tissue samples

Surgeons will collect the tissue samples from the study participants and place them in a study labelled container. This will be handed to the research team. The experimental analysis will be carried out either immediately in a University laboratory at OCTE or in the Nuffield Dept Obs and Gyn, or after cryopreservation to enable maximum use of donated tissues. No identifying details are stored.

7.4.1. Ovarian follicles isolation

The protocol will be based on the previously described by Dolmans et al. (Dolmans, Michaux et al. 2006) to isolate small pre-antral follicles. Briefly, the cortical ovarian sample will be digested using enzymes such as trypsin or collagenase at 37 °C with gentle agitation to release follicles. Follicles are isolated from the digested tissue and cultured either in isolation, in a matrix such as alginate, in groups in either static media or using a continual perfusion system.

Follicles are incubated for several days and during this time follicle growth and morphology is assessed daily. A subsample may be analysed at various time points to determine the proportion of apoptotic cells in order to evaluate follicular viability after isolation.

7.4.2. Reaggregated ovary (RO) culture

This protocol has been modified from Eppig et al (Eppig and Wigglesworth, 2000) and established in the Williams' lab (Sheikh et al 2015). The ovarian tissue will be dissociated into single cells and the eggs can be isolated from the ovarian cells using differential plate adhesion. The cell suspension is allowed to plate down overnight, the somatic cells adhere whereas the germ cells do not. The following morning a second separation step is carried out. At this time, the floating germ cell population is removed and transferred to a new plate for a further 6-8 hrs to enable any remaining somatic cells to plate down and hence be removed from the germ cell population. The somatic cell population is also trypsinised and transferred to a new plate and allowed to plate down thus allowing any remaining germ cells to be separated from the adhesive somatic cells. After this second plating down, the two cell populations are collected, cells are counted and somatic cells and germ cells combined in the appropriate proportions. The cells are spun down to aggregate them in the presence of a binding agent (e.g the lectin LPHA). The RO pellet is then cultured overnight in medium supplemented with FBS. After the overnight culture of ROs to promote 'rounding up' of the ovary, the ROs are transferred to a new well containing culture media. The ROs are cultured for up to 28 days in medium supplemented with recombinant FSH and potentially FBS, BSA, insulin-transferrin-selenium, antibiotics and ascorbic acid. The wells are filled with ~300ul of media, and a portion is replaced with fresh media at regular intervals during the culture period (current protocols are every 2 days). The cultures are maintained at 37°C in an incubator infused with 5% CO2 and 95% air.

7.4.3. Follicle and oocyte analysis

<u>To measure follicle size</u>

Images of the follicles will be collected during the culture period for subsequent analysis of follicle diameter using ImageJ software (National Institutes of Health, USA). The oocyte diameter is visible in the follicle and is measured excluding the zona pellucida.

Sectioning and staining

<u>To study follicle morphology</u>

Follicles, ovarian tissue or ROs will be fixed (e.g. 10% buffered formalin or 4% paraformaldehyde) and

subsequently dehydrated in increasing concentrations of ethanol (10–100%) and embedded in paraffin wax. Serial 3-5 µm sections are cut and stained (e.g. hematoxylin and eosin) (Grasa et al, Christensen et al (Xu, Barrett et al. 2009). To determine if an atrum is present, sections can be stained with biotinylated-hyaluronic acid binding protein (Ploutarchou et al 2015). Follicle will be staged and counted to ascertain effectiveness of culture treatment. Other analyses can be carried out using immunohistochemistry on sections or whole follicles can be subjected to whole mount analyses and be imaged using confocal microscopy.

To study follicle function

Follicles or eggs may be subjected to various other proteomic, metabolomics or genetic analyses at various time points during or after culture to assess development and function.

7.4.4. Hormone assays

To measure the ability of follicles to secrete hormones

Hormones secreted by follicles such as progesterone, estradiol, inhibins, and anti-Müllerian hormone (AMH) can be detected in the culture media using radioimmunoasssays or ELISA kits

7.5. Definition of End of Study

The end of the study is the date when the last sample from the last participant has been analysed, and any remaining tissue destroyed.

8. SAFETY REPORTING

We believe it very unlikely our study will be associated with any serious adverse events.

However, the following considerations regarding safety are relevant to this study.

Participants, including children aged 6 and older, are already having planned surgical procedures which require hospitalisation. Study participants are only those patients who are appropriate to donate samples to us and these are:

a) Women already having planned surgery to remove ovarian tissue (whole ovaries in oophorectomy or ovarian cysts in cystectomy). In both these cases the research sample can be taken from the tissue which is already being removed (by the surgeon doing operation) and is therefore ‘left over’ after the surgery. Nothing extra will happen to these women.

b) Women having planned surgery, including caesarean section, to provide sterilisation (i.e. family completed) – the same surgeon will take a small slice of ovarian tissue (less than 5% total ovary) at the time of surgery from women who agree. This will not affect the endocrine functioning of the ovaries since 10-20% is needed for this.

c) Women and children storing their own ovarian tissue for storage for their own fertility preservation. We will ask this group of patients for their consent to donate a small extra sample for the research study. The surgeon is already doing operation. The extra amount will not affect the procedure they are already having. We are including children is this study as they are most likely to be the population having this procedure and, potentially may be the beneficiaries of this research.

The study interventions will increase their surgery time by 10 minutes only. There are extremely unlikely to be increased surgical risks above that of the planned surgical procedure.

The surgeons are not part of the research team.

Definitions of and reporting of any Serious Adverse Events are described below.

8.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

8.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures.

Reports of related and unexpected SAEs will be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the NRES report of serious adverse event form to the REC/NHS R&D and reported to the sponsor immediately (within 24 hours).

9. STATISTICS AND ANALYSIS

9.1. Description of Statistical Methods

For this study, samples will be analysed using various methods. For assessment of follicle development, tissue will be sectioned and follicles classified and quantified. Additional analyses may include proteomic, metabolomic and genomic approaches and the data generated will take the form of experimental measurements to study the factors involved in the in vitro maturation of follicles obtained from human ovarian tissue samples. Statistical analyses will be carried out using Graphpad Prism and a P value of >0.05 deemed to be significant.

9.2. Number of Participants

The number of participants is likely to small but we estimate it to be up to 50 in total.

9.3. Analysis of Endpoints

The samples of ovarian tissue will be collected during the patients' surgery. Samples are put in a labeled container for immediate analysis. No identifying patient data e.g. name, will be retained.

10. DATA MANAGEMENT

10.1. Access to Data

Direct access will be granted to authorised representatives from the Sponsor or host institution for monitoring and/or audit of the study to ensure compliance with regulations.

10.2. Data Recording and Record Keeping

Copies of consent forms will be kept securely with other study documentation. Samples will be labelled with a Study ID only (anonymous). Data from experiments will be recorded by researchers in laboratory books which are stored securely in a locked building. Data will be transcribed onto Excel spread sheets for analysis. All data is kept on secure University computers and only encrypted data sticks id used. No personal data is available to researchers about the participants.

11. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

12.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

12.5. Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by initials and a participants ID number on the CRF and any electronic database. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

12.6. Expenses and Benefits

There is no requirement for participants to attend additional visits as part of the study, but if this is the case then we will reimburse reasonable travel expenses on production of receipts, or a mileage allowance provided as appropriate.

13. FINANCE AND INSURANCE

13.1. Funding

Funding is being sought by SW and MF. Intermediate funds to support the consumables required for this project are available from SW's discretionary account at the Department of Obstetrics and Gynaecology. Salary expenses for the scientists involved are covered by individual grants or DPhil student's stipends. Initial funding was provided by two grants to Prof. Cui from BBSRC: Engineering human neural network, (BB/H008608), £3,282,711, June 10 – May 15 and Development of a high-throughput perfused three dimensional cell culture platform for stem cell study and drug testing, Taiwan Partnering Award, (BB/L003961/1), £24,991, 1 Sept 13-31 Aug 15.

13.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

14. PUBLICATION POLICY

The investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge the funding for the study. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

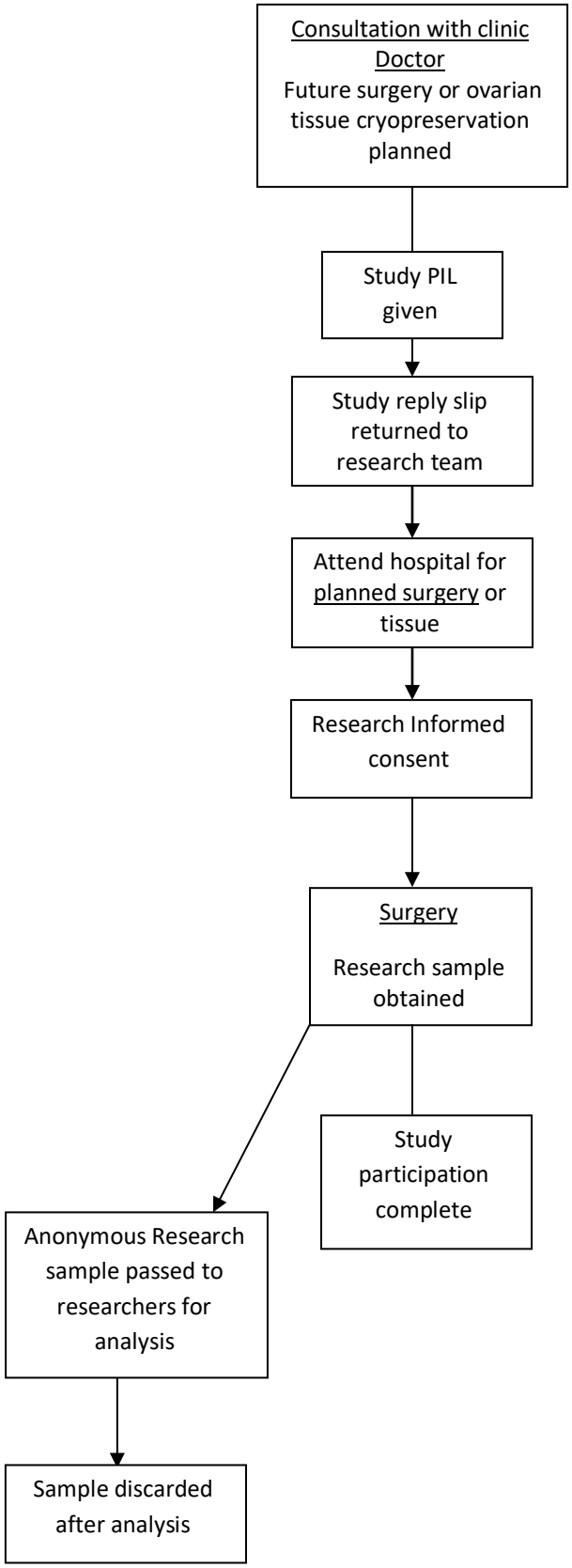
15. REFERENCES

- Abir, R., B. Fisch, S. Nitke, E. Okon, A. Raz and Z. Ben Rafael (2001). "Morphological study of fully and partially isolated early human follicles." Fertil Steril **75**(1): 141-146.
- Abir, R., S. Nitke, A. Ben-Haroush and B. Fisch (2006). "In vitro maturation of human primordial ovarian follicles: clinical significance, progress in mammals, and methods for growth evaluation." Histol Histopathol **21**(8): 887-898.
- Abir, R., P. Roizman, B. Fisch, S. Nitke, E. Okon, R. Orvieto and Z. Ben Rafael (1999). "Pilot study of isolated early human follicles cultured in collagen gels for 24 hours." Hum Reprod **14**(5): 1299-1301.
- Albertini, D. F. and S. L. Barrett (2003). "Oocyte-somatic cell communication." Reprod Suppl **61**: 49-54.
- American Cancer Society (2012). Cancer Facts & Figures 2012. . Atlanta.
- Amsden, B. and N. Turner (1999). "Diffusion characteristics of calcium alginate gels." Biotechnol Bioeng **65**(5): 605-610.
- Andersen, C. Y., M. Rosendahl, A. G. Byskov, A. Loft, C. Ottosen, M. Dueholm, K. L. Schmidt, A. N. Andersen and E. Ernst (2008). "Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue." Hum Reprod **23**(10): 2266-2272.
- Anderson, E. and D. F. Albertini (1976). "Gap junctions between the oocyte and companion follicle cells in the mammalian ovary." J Cell Biol **71**(2): 680-686.
- Borghol, N., J. Lornage, T. Blachere, A. Sophie Garret and A. Lefevre (2006). "Epigenetic status of the H19 locus in human oocytes following in vitro maturation." Genomics **87**(3): 417-426.
- Carlsson, I. B., J. E. Scott, J. A. Visser, O. Ritvos, A. P. Themmen and O. Hovatta (2006). "Anti-Mullerian hormone inhibits initiation of growth of human primordial ovarian follicles in vitro." Hum Reprod **21**(9): 2223-2227.
- Chang, T. M. (1964). "Semipermeable Microcapsules." Science **146**(3643): 524-525.
- Christensen AP, Patel SH, Grasa P, Christian HC, Williams SA (2015). Oocyte glycoproteins regulate the form and function of the**follicle** basal lamina and theca cells. Dev Biol. **401**:287-98.
- Demeestere, I., P. Simon, F. Buxant, V. Robin, S. A. Fernandez, J. Centner, A. Delbaere and Y. Englert (2006). "Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: case report." Hum Reprod **21**(8): 2010-2014.
- Demeestere, I., P. Simon, S. Emiliani, A. Delbaere and Y. Englert (2007). "Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease." Oncologist **12**(12): 1437-1442.
- Dolmans, M. M., N. Michaux, A. Camboni, B. Martinez-Madrid, A. Van Langendonckt, S. A. Nottola and J. Donnez (2006). "Evaluation of Liberase, a purified enzyme blend, for the isolation of human primordial and primary ovarian follicles." Hum Reprod **21**(2): 413-420.
- Donnez, J., M. M. Dolmans, D. Demylle, P. Jadoul, C. Pirard, J. Squifflet, B. Martinez-Madrid and A. van Langendonckt (2004). "Livebirth after orthotopic transplantation of cryopreserved ovarian tissue." Lancet **364**(9443): 1405-1410.
- Donnez, J., S. Silber, C. Y. Andersen, I. Demeestere, P. Piver, D. Meirow, A. Pellicer and M. M. Dolmans (2011). "Children born after autotransplantation of cryopreserved ovarian tissue. a review of 13 live births." Ann Med **43**(6): 437-450.
- Donnez, J., J. Squifflet, A. S. Van Eyck, D. Demylle, P. Jadoul, A. Van Langendonckt and M. M. Dolmans (2008). "Restoration of ovarian function in orthotopically transplanted cryopreserved ovarian tissue: a pilot experience." Reprod Biomed Online **16**(5): 694-704.
- Draget, K. I., G. Skjak-Braek and O. Smidsrod (1997). "Alginate based new materials." Int J Biol Macromol **21**(1-2): 47-55.
- Edwards, R. G. (1965). "Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes." Nature **208**(5008): 349-351.

- Eppig, J. J. and Wigglesworth K. (2000). Development of mouse and rat oocytes in chimeric reaggregated ovaries after interspecific exchange of somatic and germ cell components. Biol Reprod. **63**(4):1014-23.
- Galdones, E., L. C. Shea and T. K. Woodruff (2011). Three-dimensional in vitro ovarian follicle culture. In "Human assisted reproductive technology: future trends in laboratory and clinical practice" Editors D. K. Gardner, B. R. M. B. Rizk, and T. Falcone.
- Grasa P, Ploutarchou P, Williams SA (2015). Oocytes lacking O-glycans alter follicle development and increase fertility by increasing follicle FSH sensitivity, decreasing apoptosis, and modifying GDF9:BMP15 expression. FASEB J. **29**:525-39.
- Heise, R. B., J. E. J. Smitz and J. G. Thompson (2011). Current status and future trends of the clinical practice of human oocyte in vitro maturation. In "Human assisted reproductive technology: future trends in laboratory and clinical practice" Editors D. K. Gardner, B. R. M. B. Rizk, and T. Falcone.
- Heise, M., R. Koepsel, A. J. Russell and E. A. McGee (2005). "Calcium alginate microencapsulation of ovarian follicles impacts FSH delivery and follicle morphology." Reprod Biol Endocrinol **3**: 47.
- Hovatta, O., R. Silye, R. Abir, T. Krausz and R. M. Winston (1997). "Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture." Hum Reprod **12**(5): 1032-1036.
- Hovatta, O., C. Wright, T. Krausz, K. Hardy and R. M. Winston (1999). "Human primordial, primary and secondary ovarian follicles in long-term culture: effect of partial isolation." Hum Reprod **14**(10): 2519-2524.
- Kim, B. S. and D. J. Mooney (1998). "Development of biocompatible synthetic extracellular matrices for tissue engineering." Trends Biotechnol **16**(5): 224-230.
- Kreeger, P. K., J. W. Deck, T. K. Woodruff and L. D. Shea (2006). "The in vitro regulation of ovarian follicle development using alginate-extracellular matrix gels." Biomaterials **27**(5): 714-723.
- Kreeger, P. K., N. N. Fernandes, T. K. Woodruff and L. D. Shea (2005). "Regulation of mouse follicle development by follicle-stimulating hormone in a three-dimensional in vitro culture system is dependent on follicle stage and dose." Biol Reprod **73**(5): 942-950.
- Li, Y., H. L. Feng, Y. J. Cao, G. J. Zheng, Y. Yang, S. Mullen, J. K. Critser and Z. J. Chen (2006). "Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro." Fertil Steril **85**(4): 827-832.
- Meirow, D., J. Levron, T. Eldar-Geva, I. Hardan, E. Fridman, Z. Yemini and J. Dor (2007). "Monitoring the ovaries after autotransplantation of cryopreserved ovarian tissue: endocrine studies, in vitro fertilization cycles, and live birth." Fertil Steril **87**(2): 418 e417-418 e415.
- Meirow, D., J. Levron, T. Eldar-Geva, I. Hardan, E. Fridman, Y. Zalel, E. Schiff and J. Dor (2005). "Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy." N Engl J Med **353**(3): 318-321.
- Monniaux, D., C. Huet-Calderwood, F. Le Bellego, S. Fabre, P. Monget and D. A. Calderwood (2006). "Integrins in the ovary." Semin Reprod Med **24**(4): 251-261.
- Murray, A. A., R. G. Gosden, V. Allison and N. Spears (1998). "Effect of androgens on the development of mouse follicles growing in vitro." J Reprod Fertil **113**(1): 27-33.
- Nebel, R. L., J. H. Bame, R. G. Saacke and F. Lim (1985). "Microencapsulation of bovine spermatozoa." J Anim Sci **60**(6): 1631-1639.
- Oktay, K., E. Buyuk, L. Veeck, N. Zaninovic, K. Xu, T. Takeuchi, M. Opsahl and Z. Rosenwaks (2004). "Embryo development after heterotopic transplantation of cryopreserved ovarian tissue." Lancet **363**(9412): 837-840.
- Oktay, K., H. Newton and R. G. Gosden (2000). "Transplantation of cryopreserved human ovarian tissue results in follicle growth initiation in SCID mice." Fertil Steril **73**(3): 599-603.
- Oktay, K., D. Nugent, H. Newton, O. Salha, P. Chatterjee and R. G. Gosden (1997). "Isolation and characterization of primordial follicles from fresh and cryopreserved human ovarian tissue." Fertil Steril **67**(3): 481-486.

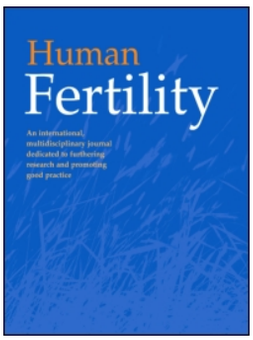
- Pangas, S. A., H. Saudye, L. D. Shea and T. K. Woodruff (2003). "Novel approach for the three-dimensional culture of granulosa cell-oocyte complexes." Tissue Eng **9**(5): 1013-1021.
- Ploutarchou P, Melo P, Day AJ, Milner CM, Williams SA (2015). Molecular analysis of the cumulus matrix: insights from mice with O-glycan-deficient oocytes. Reproduction. **149**:533-43.
- Radford, J. A., B. A. Lieberman, D. R. Brison, A. R. Smith, J. D. Critchlow, S. A. Russell, A. J. Watson, J. A. Clayton, M. Harris, R. G. Gosden and S. M. Shalet (2001). "Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma." Lancet **357**(9263): 1172-1175.
- Reynaud, K., R. Cortvrindt, J. Smitz and M. A. Driancourt (2000). "Effects of Kit Ligand and anti-Kit antibody on growth of cultured mouse preantral follicles." Mol Reprod Dev **56**(4): 483-494.
- Rosendahl, M., A. Loft, A. G. Byskov, S. Ziebe, K. T. Schmidt, A. N. Andersen, C. Ottosen and C. Y. Andersen (2006). "Biochemical pregnancy after fertilization of an oocyte aspirated from a heterotopic autotransplant of cryopreserved ovarian tissue: case report." Hum Reprod **21**(8): 2006-2009.
- Roy, S. K. and B. J. Treacy (1993). "Isolation and long-term culture of human preantral follicles." Fertil Steril **59**(4): 783-790.
- Schmidt, K. L., C. Y. Andersen, A. Loft, A. G. Byskov, E. Ernst and A. N. Andersen (2005). "Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation." Hum Reprod **20**(12): 3539-3546.
- Sheikh, S., H. Kaune, A. Deleva and S.A. Williams (2015). The role of the oocyte is investigated in Premature Ovarian Failure using the Reaggregated Ovarian Pellet technique Society for Reproduction and Fertility Abstract.
- Smidsrod, O. and G. Skjak-Braek (1990). "Alginate as immobilization matrix for cells." Trends Biotechnol **8**(3): 71-78.
- Smitz, J. and R. Cortvrindt (1998). "Follicle culture after ovarian cryostorage." Maturitas **30**(2): 171-179.
- Telfer, E. E., M. McLaughlin, C. Ding and K. J. Thong (2008). "A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin." Hum Reprod **23**(5): 1151-1158.
- Torre, M. L., M. Faustini, K. M. Attilio and D. Vigo (2007). "Cell encapsulation in mammal reproduction." Recent Pat Drug Deliv Formul **1**(1): 81-85.
- Tryde Schmidt, K. L., C. Yding Andersen, J. Starup, A. Loft, A. G. Byskov and A. Nyboe Andersen (2004). "Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer - follicular growth, steroid production and oocyte retrieval." Reprod Biomed Online **8**(4): 448-453.
- West, E. R., M. Xu, T. K. Woodruff and L. D. Shea (2007). "Physical properties of alginate hydrogels and their effects on in vitro follicle development." Biomaterials **28**(30): 4439-4448.
- Xu, M., S. L. Barrett, E. West-Farrell, L. A. Kondapalli, S. E. Kiesewetter, L. D. Shea and T. K. Woodruff (2009). "In vitro grown human ovarian follicles from cancer patients support oocyte growth." Hum Reprod **24**(10): 2531-2540.
- Xu, M., P. K. Kreeger, L. D. Shea and T. K. Woodruff (2006). "Tissue-engineered follicles produce live, fertile offspring." Tissue Eng **12**(10): 2739-2746.
- Xu, M., E. West, L. D. Shea and T. K. Woodruff (2006). "Identification of a stage-specific permissive in vitro culture environment for follicle growth and oocyte development." Biol Reprod **75**(6): 916-923.

16. APPENDIX A: STUDY FLOW CHART



17. APPENDIX B: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	1.1	24/02/2014	Initial Version	N/A
2	2.0	27/01/2016	Suzannah Williams	1. New CI 2. Updated culture protocols and more general analysis



Taylor & Francis
Taylor & Francis Group

Human Fertility

an international, multidisciplinary journal dedicated to furthering research and promoting good practice

ISSN: 1464-7273 (Print) 1742-8149 (Online) Journal homepage: https://www.tandfonline.com/loi/ihuf20

Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation

Charlotte A. Walker, Briet D. Bjarkadottir, Muhammad Fatum, Sheila Lane & Suzannah A. Williams

To cite this article: Charlotte A. Walker, Briet D. Bjarkadottir, Muhammad Fatum, Sheila Lane & Suzannah A. Williams (2019): Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation, Human Fertility, DOI: 10.1080/14647273.2019.1616118

To link to this article: https://doi.org/10.1080/14647273.2019.1616118

HUMAN FERTILITY
https://doi.org/10.1080/14647273.2019.1616118

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation

Charlotte A. Walker[a], Briet D. Bjarkadottir[a], Muhammad Fatum[a,b], Sheila Lane[c] and Suzannah A. Williams[a]

[a]Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK; [b]Oxford Fertility, Institute of Reproductive Sciences, Oxford, UK; [c]Department of Paediatric Oncology and Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

ABSTRACT

This study investigated how follicle health and development in human ovarian tissue cryopreserved for fertility preservation varied between patients before and after 6 d of *in vitro* culture. Ovarian tissue from 12 patients (9–25 years) was used. In 3 patients, a 1hr neutral red (NR) incubation was used to identify tissues with viable follicles. Tissues were fixed, sectioned and follicles staged and graded for health. Inter-patient differences were observed in the non-cultured tissue in the number of both healthy follicles ($p = 0.005$) and growing follicles ($p = 0.005$). After culture there was significant variation in the number of transitional, primary and secondary follicles between patients ($p < 0.001$). Asymmetric primary follicles with a single complete layer of granulosa cells plus two or more additional partial layers were 5.5 times more likely to be observed in cultured compared to non-cultured tissue ($p = 0.0063$). Non-cultured ($p = 0.0125$) and cultured ($p < 0.001$) tissue selected using NR had more healthy follicles compared to tissue not selected using NR. Non-cultured and cultured tissue selected using NR had more healthy follicles compared to tissue not selected using NR ($p = 0.0125$; $p < 0.001$). We demonstrate that inter-patient variation exists in the health and development of follicles before and after culture. Culture systems need to be optimized to support cryopreserved ovarian tissue and these findings should prompt researchers to consider patient variation when evaluating culture systems.

ARTICLE HISTORY
Received 26 November 2018
Accepted 7 March 2019

KEYWORDS
Cryopreservation; ovary; follicle; human; *in vitro* growth; cancer

Introduction

Cryopreservation of ovarian cortical tissue has been established as a successful method for preserving fertility in patients for whom traditional approaches, such as storage of mature eggs or embryos, are not possible. This includes pre-pubertal girls and women for whom cancer treatment cannot be delayed (Anderson, Wallace, & Telfer, 2017). Growth of follicles from this tissue *in vitro* is emerging as an alternative method to circumvent some of the limitations associated with transplantation (Nisolle, Casanas-Roux, Qu, Motta, & Donnez, 2000).

The overall goal of *in vitro* follicle growth is to generate systems that support development and maturation of a competent human egg for use by women at risk of premature ovarian insufficiency (De Vos, Smitz, & Woodruff, 2014; McLaughlin, Albertini, Wallace, Anderson, & Telfer, 2018; Xiao et al., 2015). For humans, an *in vitro* system capable of generating metaphase II oocytes from fresh cortical tissue has recently been described using a three-step culture method prior to *in vitro* maturation (McLaughlin et al., 2018). However, culture systems aiming to support the initiation of follicle growth and early development have predominantly used fresh tissue from healthy women undergoing gynaecological operations whose biology may differ from those who may benefit from fertility preservation. To date, there is no data regarding the variation in health and development of follicles *in vitro* from tissue cryopreserved for fertility preservation in women undergoing gonadotoxic treatments, despite the high importance of this for developing techniques for fertility restoration.

Follicle distribution in the cortex of human ovaries is extremely heterogeneous. The density of primordial follicles between different pieces of cortex from the same ovary can vary by greater than two orders of magnitude (Schmidt, Byskov, Nyboe Andersen, Muller,

CONTACT Suzannah A Williams suzannah.williams@wrh.ox.ac.uk Nuffield Department of Women's and Reproductive Health, University of Oxford, Level 3, Women's Centre, John Radcliffe Hospital, Oxford OX3 9DU, UK

& Yding Andersen, 2003). This creates challenges in identifying cortical tissue fragments with follicles for culture. Neutral Red (NR) is a weak cationic supravital dye that is soluble in water and has been used in cytotoxicity studies as a marker of cell viability (Allison & Young, 1964; Borenfreund & Puerner, 1985). It has been demonstrated to effectively label viable follicles within ovine cortical tissue and has subsequently been used to determine follicular density of fresh human cortical tissue or medulla by incubating the tissue for 4 h before visualization (Chambers, Gosden, Yap, & Picton, 2010; Kristensen et al., 2018). It remains to be investigated whether this dye can be incorporated into the workflow of human cortical strip culture without the use of a 4-h incubation to allow visualization of follicles in fresh tissue before culture.

The aims of this study were to determine: (i) how the health and developmental stage of follicles differs between women who have cryopreserved cortical tissue for fertility preservation; (ii) whether the *in vitro* developmental capacity of follicles from cryopreserved tissue varies between them; and (iii) whether incubating cortical tissue with NR for a short period of time to identify tissue with viable follicles could improve the number of viable follicles after 6 d of culture.

Materials and methods

Ethical approval

The use of human tissue was approved by Health Research Authority South Central – Oxford B Research Ethics Committee (REC reference: 14/SC/0041).

Ovarian tissue collection

Cryopreserved ovarian tissue was obtained from twelve patients aged 9–25 years. All were undergoing ovarian tissue cryopreservation, as a fertility preservation measure due to malignancy or blood disorder. None had received chemotherapy or radiation treatment prior to ovarian tissue cryopreservation. As part of the consent process, permission to use tissue in research had been obtained.

Chemicals and consumables

Leibovitz L-15 medium, McCoy's 5A (modified) HEPES buffered medium, L-glutamine and ascorbic acid were obtained from Thermo Fisher (Paisley, UK). Human serum albumin (HSA), ITS liquid media supplement (100×), sucrose, ethylene glycol, sodium pyruvate, NR, Bouin's solution and Whatman Nuclepore membranes were obtained from Sigma Aldrich (Poole, UK). Recombinant human follicle stimulating hormone (FSH; Gonal-F) was obtained from Merck Serono (Feltham, UK). Corning Costar tissue culture treated 24-well plates were obtained from Scientific Laboratory Supplies (Nottingham, UK).

Cryoprotection and cryopreservation

Ovarian tissue cryopreservation was performed by the Oxford Cell and Tissue Biobank. Cortical tissue cut into cortical strips (~2 × 1 × 5 mm) were placed into cryovials containing 1 mL of cryoprotectant medium (1.5 M ethylene glycol, 0.1 M sucrose, 10% (v/v) serum substitute supplement in L-15 medium) and incubated for 1 h at 4 °C before being frozen using a controlled rate slow freezer (IceCube 15M; SY-LAB, Norcross, GA). Following cryopreservation, the vials were stored in vapour phase liquid nitrogen.

Tissue thawing and cortical strip culture

Cryovials were held at room temperature for approximately 1 min before being immersed in a 30 °C water bath for 3 min. Cortical strips were washed through three thawing solutions containing a reversed ethylene glycol gradient (1, 0.5 and 0 M), 0.1 M sucrose and 3 mg/mL HSA in Leibovitz L-15 medium for 5 min and transferred to a petri dish containing Dissection Medium (3 mg/mL HSA, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine and 2 mM sodium pyruvate in L-15 medium). Tissue was mechanically chopped using the McIlwain tissue chopper, and further cut manually using a scalpel and forceps into ~0.5 × 0.5 × 0.25 mm pieces.

Tissue pieces of uniform size were distributed randomly and evenly between wells of a 24-well plate with excess remnant tissue discarded. A portion of tissue fragments was fixed overnight in Bouin's fixative as a non-cultured control. Tissue was cultured on a polycarbonate membrane (13 mm diameter, 8 μm pore size) floating on 1 mL of culture medium in a 24-well culture plate; culture medium contained McCoy's 5A supplemented with 1 mg/mL HSA, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 10 μg/mL insulin, 5.5 μg/mL transferrin, 5 ng/mL selenium, 50 μg/mL ascorbic acid and 12.5 IU/L recombinant human FSH. Cortical tissue was cultured for 6 d at 37 °C under 5% CO_2 in air, with half the medium being replaced with fresh medium every other day. Following the culture period, the tissue pieces were fixed overnight in

Table 1. Characteristics of patients diagnosed with malignant disease or blood disorder who underwent ovarian tissue cryopreservation for fertility preservation.

Patient	Age (years)	Post-pubertal	Regular periods	Diagnosis	Prior treatment	Time from surgery to cryopreservation
A	9	No	N/A	Medulloblastoma	None	3 h 16 min
B	11	No	N/A	Sickle cell	None	4 h 19 min
C	16	No	N/A	Osteosarcoma	None	5 h 20 min
D	16	Yes	Unknown	Ewing's sarcoma	None	5 h 10 min
E	17	Yes	Yes	Ewing's sarcoma	None	2 h 05 min
F	18	Yes	Unknown	Sickle cell	None	3 h 57 min
G	19	Yes	Unknown	Low-grade serous adenocarcinoma	None	6 h 15 min
H	22	Yes	Unknown[a]	Burkitt lymphoma stage 1A	None	4 h 18 min
I	22	Yes	Unknown	Atypical teratoid Rhabdoid tumour (Grade IV)	None	5 h 00 min
J	23	Yes	Yes	Breast cancer, grade 3, oestrogen positive, non-metastatic	None	3 h 05 min
K	23	Yes	Unknown[a]	Hodgkin lymphoma	None	4 h 20 min
L	25	Yes	Yes	Cervical cancer	None	3 h 32 min

[a]Patient taking the oral contraceptive pill.

Bouin's solution and stored in 70% ethanol until they were processed.

Neutral red (NR) visualization

To identify fragments containing follicles, after processing cortical tissue with the tissue chopper, fragments from patients E, I and L (Table 1) were incubated in NR (25 μg/mL in dissection medium) at room temperature for 1 h while the tissue was cut (as in the standard protocol). Allocation of tissue between groups was performed without a microscope to blind the researcher to the degree of staining and detail of the tissue pieces.

Histological analysis

The fixed ovarian tissue was dehydrated through a graded series of ethanol (70, 80, 95, 3 × 100%), cleared in xylene and embedded in paraffin wax at 64 °C for three hours. The wax-embedded tissue was serially sectioned at 5 μm and stained with haematoxylin and eosin.

Follicles were staged based on criteria described by Gougeon (1986); primordial (single layer of flattened pre-granulosa cells), transitional (single layer with at least one cuboidal granulosa cell), primary (complete layer of cuboidal granulosa cells) and secondary, two or more complete layers of cuboidal granulosa cells (Figure 1). Where follicles had a single complete layer of cuboidal cells, the number of any additional partial layers of granulosa cells was recorded. Non-growing follicles were those at the primordial stage. Transitional, primary and secondary follicles were classed as growing. Granulosa cell and oocyte pyknosis, and shrinkage of ooplasm were selected as markers for follicle health based on the existing literature and the ease of assessing health factors in a non-biased manner (Gougeon, 1986). Healthy follicles had a non-pyknotic non-shrunken oocyte with non-pyknotic granulosa cells, degenerating follicles had one of the above factors, while follicles were classified as atretic if they had both an oocyte with a pyknotic nucleus and pyknotic granulosa cells (Figure 2). All tissue sections were analysed for the presence of follicles, and only follicles with a visible nucleolus or a clearly defined nuclear membrane were assessed, to avoid double-counting. A blinded researcher performed follicle counts and assessment; a second blinded researcher confirmed repeatability.

Calculation of follicle density

To determine the volume of the tissue, the area of every 12th tissue section was measured using ImageJ 1.46r (National Institutes of Health, Bethesda, MD). Follicle density was determined by dividing the total number of follicles counted in a tissue sample by the tissue volume.

Statistical analysis and modelling

All statistical analyses were performed using R statistical software version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria). Statistical analysis was performed using Fisher's Exact Test. Tests adjusting for patient variation used the lme4 package in R (Bates, Mächler, Bolker, & Walker, 2015). All logistic regressions were offset for tissue volume and included patient as a random effect. Data are presented as mean or as odds ratio with 95% confidence intervals where available and statistical significance was defined

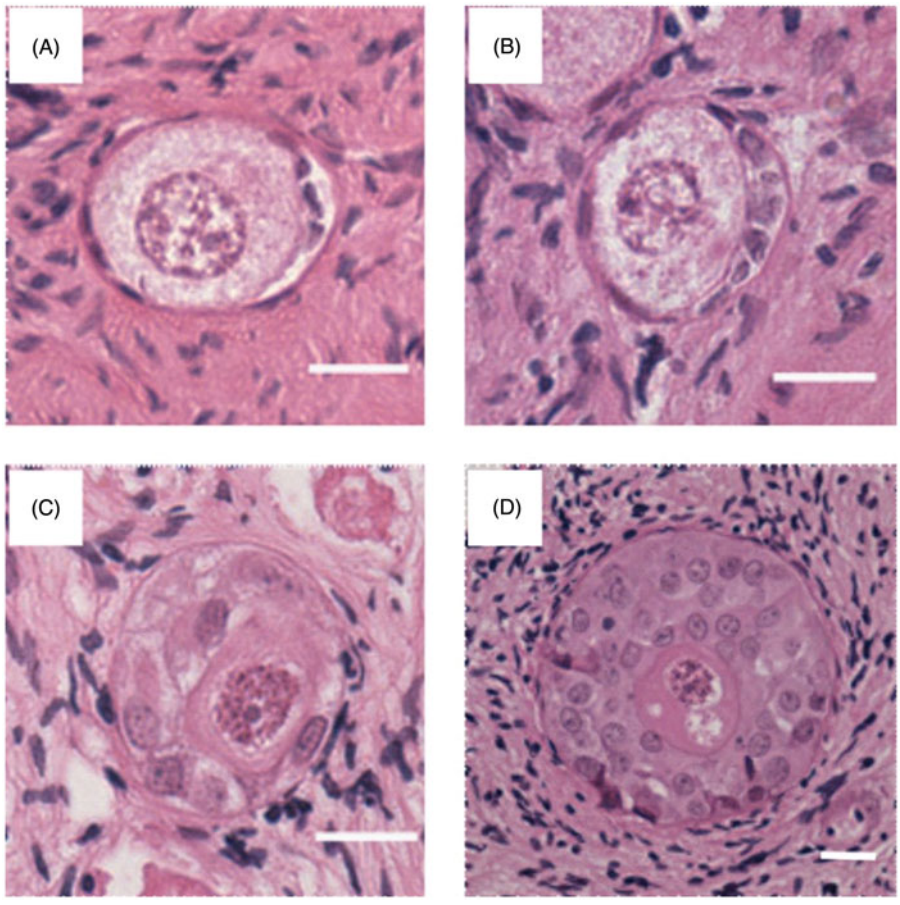


Figure 1. Developmental staging of human follicles from cryopreserved ovarian tissue from patients diagnosed with malignant disease or blood disorder. Representative images of follicles in cryopreserved-thawed ovarian tissue. Tissue was fixed in Bouin's, embedded and stained with haematoxylin and eosin. Follicles were staged as: (A) primordial with a single layer of flattened pre-granulosa cells; (B) transitional with a single layer of at least one cuboidal granulosa cell; (C) primary with a complete layer of cuboidal granulosa cells; or (D) secondary with two or more complete layers of cuboidal granulosa cells. Scale bar = 20 μm.

as $p < 0.05$. Due to the small number of secondary follicles observed, no statistical analysis was performed on this cohort.

Results

Patient characteristics

Nine of the twelve patients were confirmed as having gone through puberty, the remaining three had not. Of the nine post-pubertal patients, information about menstrual cycle was available for three who were reported as having regular cycles (Table 1). Two patients, where information about their menstrual cycle was unavailable, were taking the oral contraceptive pill. The mean age (±SEM) of patients who had gone through puberty was significantly higher than for those who had not (20.56 ± 1.04 *vs.* 12 ± 2.08 years, respectively; $p < 0.01$).

There was no difference in the time from tissue procurement to cryopreservation between the pre- and post-pubertal groups. The mean time from completion of surgery to start of cryopreservation was 4 h 13 ± 19 min (Table 1).

Follicular density of tissue post-cryopreservation from the 12 patients varied from 18.10 to 448.43 follicles/mm^3 between patient samples (Figure 3) and all cortical strips contained follicles. Despite the small sample of tissue and the known heterogeneity of follicle distribution in the ovary, there was a significant inverse correlation between follicular density and age ($r = -0.62$, $p < 0.05$).

Number of growing follicles in non-cultured cryopreserved tissue varies between patients

Follicles were observed in non-cultured tissue from 11 of the 12 patient samples. Non-growing follicles were

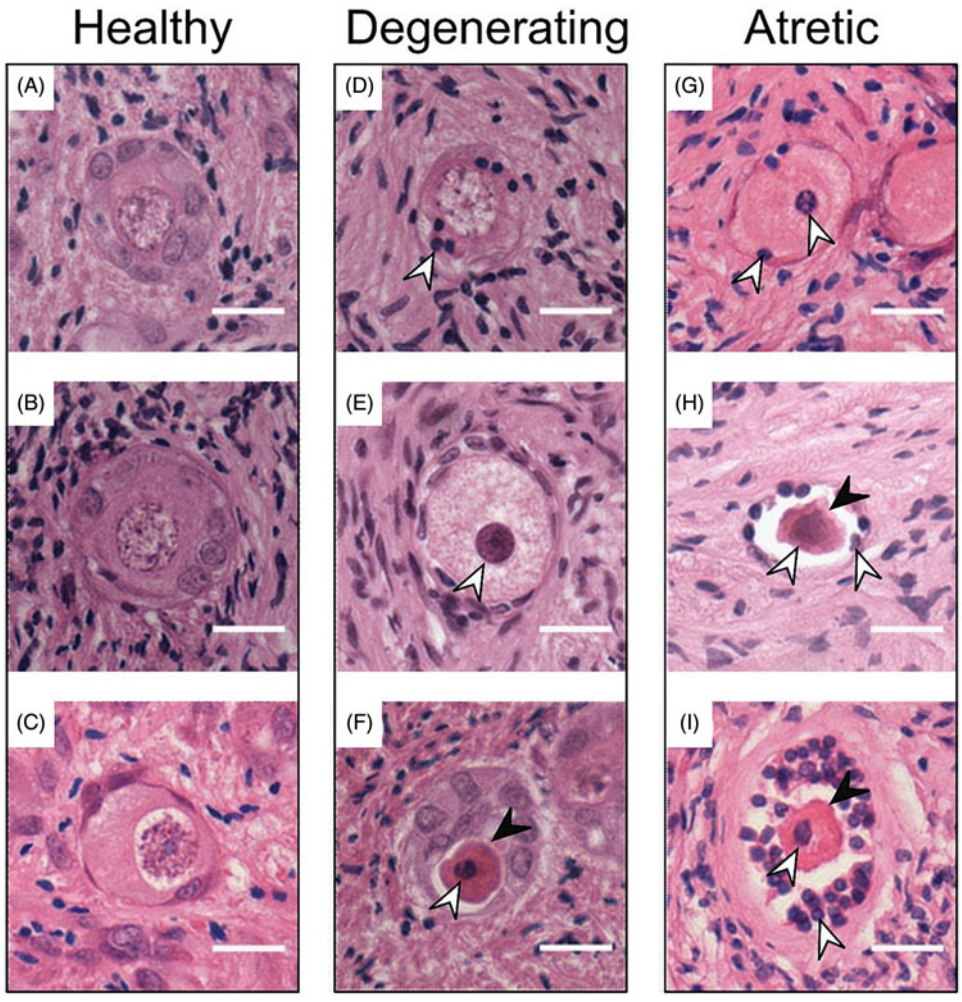


Figure 2. Classification of follicle health in human follicles from cryopreserved ovarian tissue from patients diagnosed with malignant disease or blood disorder. Follicle health was assessed based on the presence or absence of pyknotic granulosa cells, a pyknotic oocyte and a shrunken ooplasm: Images A–C show healthy follicles with healthy granulosa cells, a non-pyknotic and non-shrunken oocyte whereas degenerating follicles had either (D) pyknotic granulosa cells (white arrowhead), (E) a pyknotic oocyte (white arrowhead) with normal ooplasm or (F) a pyknotic oocyte (white arrowhead) with a shrunken ooplasm (black arrowhead) and normal granulosa cells. Atretic follicles had both pyknotic granulosa cells and a pyknotic oocyte (white arrowheads), without shrunken ooplasm (G), or with shrunken ooplasm (black arrowheads, H,I). Scale bar = 20 μm.

those classified as primordial based on morphology (Figure 1(A)), growing follicles were those classified as transitional, primary, or secondary (Figure 1(B–D)). The number of growing compared to non-growing follicles in uncultured tissue varied significantly based on patient and can be seen in Figure 4(A) ($p < 0.001$). Non-growing follicles were the dominant population in non-cultured tissue for five patient samples (patients A, B, E, H and I), an equal percentage of growing and non-growing were seen in two patient samples (patients C and D), and the percentage of growing follicles dominated in four patient samples (patients G, J, K and L). Interestingly, only growing follicles were observed in non-cultured tissue from patient K, however, as only three follicles were seen in this tissue (Table 2) it may be due to the small sample size.

Follicle health in non-cultured cryopreserved tissue varies between patients

Follicles were graded as being healthy, degenerating or atretic based on the presence or absence of pyknotic granulosa cells, a pyknotic oocyte and shrunken ooplasm (Figure 2). There was significant variation between patient samples in the percentage of healthy follicles in non-cultured tissue (Figure 4(B); $p < 0.001$).

In three patient samples, tissue fragments were preferentially selected for non-culture or culture based on visualization of follicles using the vital dye NR.

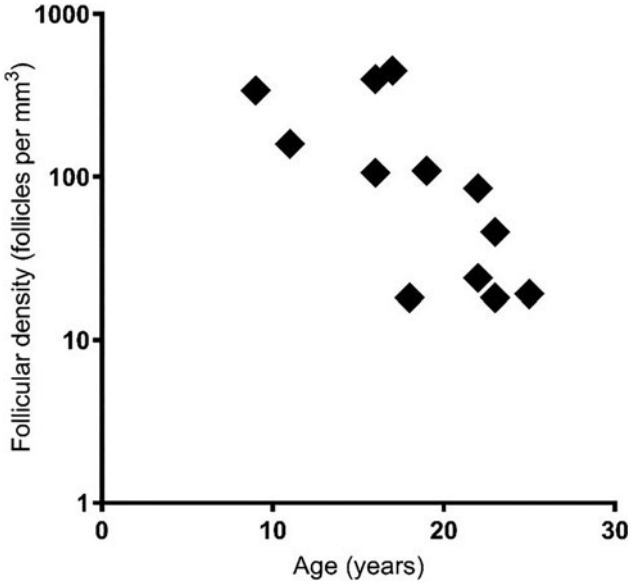


Figure 3. The density of follicles in relation to age in cryopreserved ovarian cortical tissue from 12 patients diagnosed with a malignant disease or blood disorder.

Tissues from these samples (patients E, I and J) showed a similar percentage of healthy follicles in the primordial and growing follicle populations. This was in contrast to the nine patient samples in which NR was not used as in five of these, the percentage of healthy follicles was greater in the primordial population compared to the growing population (Figure 4(C,D)).

The probability of a follicle being healthy in non-cultured tissue did not differ between pre- and post-pubertal patients and was unaffected by the time from surgery to cryopreservation.

Follicle health, but not development is greater in non-cultured tissue selected by neutral red staining

An example of a follicle with NR staining can be seen in Figure 5(A). In non-cultured tissue where NR was used to select tissue fragments as described above, the odds of a follicle being healthy was over 11 times that for follicles from tissue where NR was not used (Figure 5(B); OR = 11.4, 95% CI (1.7–77.4); $p = 0.0125$). There was a smaller percentage of growing follicles in tissue selected using NR though this was not significant (Figure 5(B)).

Cultured cryopreserved tissue has a dominant population of growing follicles in all patients

Overall, culture resulted in a significant change in the non-growing and growing follicle populations, with the odds of observing a growing follicle in cultured tissue being 52 times that of non-cultured tissue (OR = 52.44, 95% CI (37.05–76.41), $p < 0.0001$).

The health and development of follicles in tissue from each patient samples are detailed in Figure 6. There was no difference in the percentage of follicles growing following culture (Figure 6(A)), however, there was variation within the growing population in the proportion of follicles at the transitional, primary and secondary stages (Figure 6(B)). Primary follicles were the dominant growing follicles in all patient samples except for patient K and patient B where 50% of follicles were transitional and 50% were primary. The number of secondary follicles observed after 6 d of culture was low compared to transitional and primary follicles and represented at most 9% of the total follicles in cultured tissue (Figure 6(B); Patient C). Between 8 and 80% of follicles were multi-laminar follicles with more than one layer of granulosa cells (Figure 6(C)). The percentage of growing follicles in tissue after 6 d of culture was not different between fragments that had been selected based on NR staining and those that had not been selected based on NR (Figure 6(F)).

Neutral red selected tissue has superior follicle health post-culture

Despite significant activation and development of follicles in cultured tissue, overall follicle survival in cultured tissue was low. Following 6 d of culture, all follicles from tissue not selected using NR showed some sign of degeneration (Figure 6(E)), however, this is not surprising as 78.08% of follicles in this cohort showed some sign of degeneration in non-cultured cryopreserved tissue (Figure 5). In tissue selected by NR staining, 18.14% ($n = 612$; 3 patients) of follicles were healthy after 6 d of culture (Figure 6(E)). Since 60.39% of follicles were healthy in non-cultured tissue selected using NR (Figure 5), this meant that follicles in these tissues were over 13 times more likely to be healthy in non-cultured compared to cultured tissue (OR= 13.67, 95% CI (10.04–18.91), $p < 0.0001$). The odds of a follicle from NR selected tissue being healthy after 6 d in culture were 26 times that of a follicle from tissue where NR was not used to select fragments for culture (Figure 6(E); OR = 108.9, 95% CI (12.3–962.8), $p < 0.001$).

Follicles grown in culture have asymmetric granulosa cell distribution

In cultured tissue, primary follicles were 3.2 times more likely to have at least one additional partial

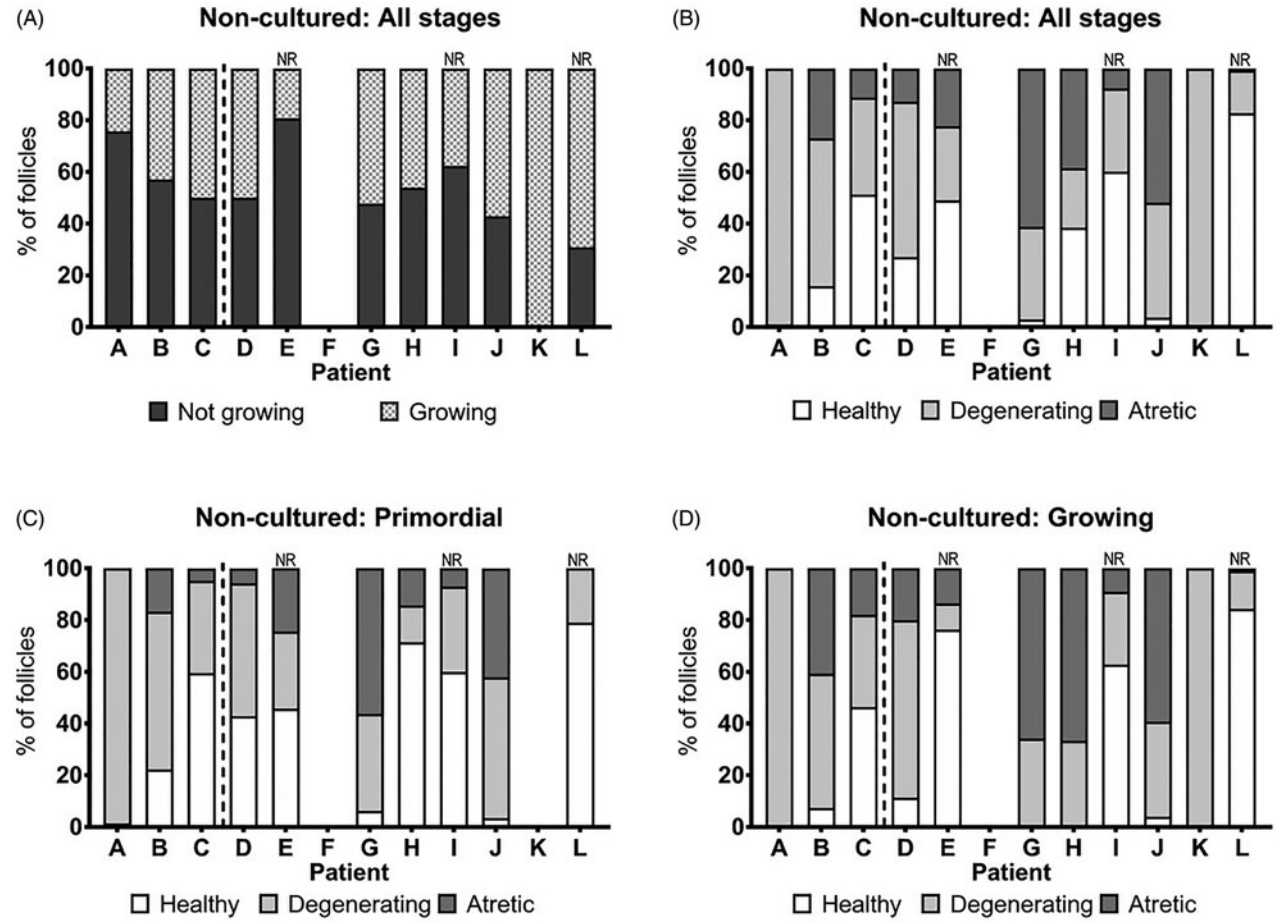


Figure 4. Variation between patients in the percentage of growing and healthy follicles in non-cultured cryopreserved human ovarian tissue from patients diagnosed with malignant disease or blood disorder. Patients are listed by ascending age with the dashed line indicating the split between those who were not post-pubertal (patients A–C) and those who were post-pubertal (patients D–L). Significant variation in the number of growing follicles was observed in non-cultured cryopreserved ovarian cortical tissue from all patients with follicles (A) ($p < 0.001$, Fisher's Exact Test); no follicles were observed in non-cultured tissue from patient F. The number of healthy (no morphological evidence of degeneration), degenerating (presence of either a pyknotic oocyte, pyknotic granulosa cells or shrunken ooplasm), and atretic (presence of both pyknotic oocyte and pyknotic granulosa cells) varied significantly between patients (B) ($p < 0.001$, Fisher's Exact Test). Images (C,D) show the percentage of healthy primordial follicles was greater than that of the growing follicle population in non-cultured tissue, with the exception of those follicles stained with neutral red (NR; patients E, I and L) which showed a similar percentage of healthy follicles in the primordial and growing follicle populations. NR: neutral red staining used to select tissue.

Table 2. Number of follicles observed in cultured and non-cultured cryopreserved human ovarian tissue.

Patient	Non-cultured	Cultured	Total	Selected using neutral red
A	99	27	126	–
B	63[a]	85	148	–
C	168[a]	67[a]	235	–
D	70	58	128	–
E	1224	489[a]	1713	Yes
F	0	14	14	–
G	67[a]	68	135	–
H	13	14[a]	27	–
I	874	90	964	Yes
J	133[a]	15[a]	148	–
K	3[a]	12	15	–
L	139	33[a]	172	Yes

[a]Secondary follicles present.

granulosa cell layer compared to non-cultured primary follicles (OR = 3.17, 95% CI (1.83–5.80), $p < 0.001$; Figure 7(A)). Asymmetric follicles, primary follicles with two or more partial layers of granulosa cells and only one complete layer of granulosa cells, were observed in eight of the cultured patient samples (Figure 7(C,D)). By contrast in non-cultured tissue, only two asymmetric primary follicles were observed, each in fragments of tissue from different patients (Figure 7(A)). The odds of a primary follicle being asymmetric in cultured tissue was 5.5 times that of non-cultured tissue (OR = 5.46, 95% CI (1.40–46.92), $p = 0.0063$).

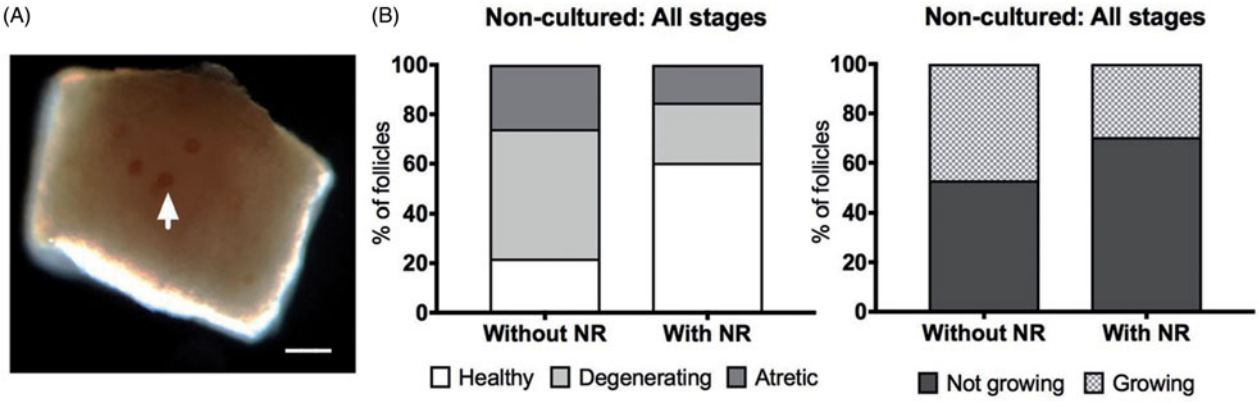


Figure 5. Use of neutral red facilitates selection of tissue fragments containing viable follicles in cryopreserved ovarian tissue from patients diagnosed with malignant disease or blood disorder. Cortical tissue incubated in neutral red NR for 1 h revealed viable follicles stained red shown in image (A) (white arrowhead). Thawed cryopreserved tissue fragments with follicles identified by NR staining for 1 h ($n = 3$ patients) contained significantly more healthy follicles compared to tissue where NR was not used (B) ($n = 9$ patients) ($p < 0.02$, OR = 11.4, 95% CI (1.7–77.4), logistic regression adjusted for tissue volume with patient as a random effect). There was no difference in the number of growing follicles in non-cultured tissue selected by NR staining compared to tissue where NR was not used. Scale bar = 100 μm.

Discussion

This study is, to our knowledge, the first to evaluate the patient-specific health and development of follicles following *in vitro* culture of cortical tissue cryopreserved for fertility preservation. Furthermore, we describe a protocol for human cortical strip culture utilizing NR to identify fragments with viable follicles for culture.

Our results highlight wide variation between patients in the health and developmental stage of follicles in cryopreserved ovarian cortical tissue. Early studies looking at the viability of ovarian cortical tissue cryopreserved in humans focused on success of xenotransplantation studies to demonstrate function without accompanying histological assessment. Variation and lack of clarity in morphological criteria for atresia between studies, as well as differing methods of cryoprotection, has made it challenging to define a baseline for cryopreservation-derived follicle degeneration. Here, we describe the morphological criteria assessed, providing images of each variation of follicle health we observed. Variation in follicle health post-thaw may be tied to follicle health in fresh tissue as despite finding no difference between fresh and frozen-thawed follicles, 27% of follicles in fresh tissue showed multiple signs of atresia; eosinophilia of the ooplasm, contraction and clumping of the chromatin material, and wrinkling of the nuclear membrane (Hovatta et al., 1996).

We found the use of just 1 h of NR incubation effective and easy to implement within the culture workflow with follicles identified in thin cortical fragments by their red staining. When tissue with viable follicles was selected by NR-staining, a significantly greater number of follicles in non-cultured tissue had a healthy morphology (60% compared to 22%, respectively). Our findings support those from Kristensen et al. (2018) who used a 4 h NR incubation to demonstrate the specificity of NR for viable follicles in cortical tissue from women undergoing fertility preservation before gonadotoxic treatment. Additionally, our results demonstrate that a shorter NR incubation period is adequate to identify tissue fragments with viable follicles, enhancing the value of such a technique in selecting tissue for culture with the aim of optimizing the proportion of healthy follicles.

In several patients, the percentage of multi-layered follicles present after 6 d of culture was equal to or greater than that published by McLaughlin, Kinnell, Anderson, and Telfer (2014) indicating both methods support equivalent follicle development. Interestingly, a significant proportion of primary follicles in cultured tissue were asymmetric, with two or more partial granulosa cell layers in addition to a single complete layer. This finding is significant as current multi-step *in vitro* protocols involve the excision of secondary follicles based on size following cortical strip culture (McLaughlin et al., 2018; Telfer, McLaughlin, Ding, & Thong, 2008). However, based on size asymmetric follicles would be indistinguishable from non-asymmetric follicles when using a dissecting, brightfield microscope. It is unclear whether the asymmetry is a result of the physical culture environment or whether it is a result of accelerated growth due to culture conditions. Furthermore, it is important to ascertain the impact of

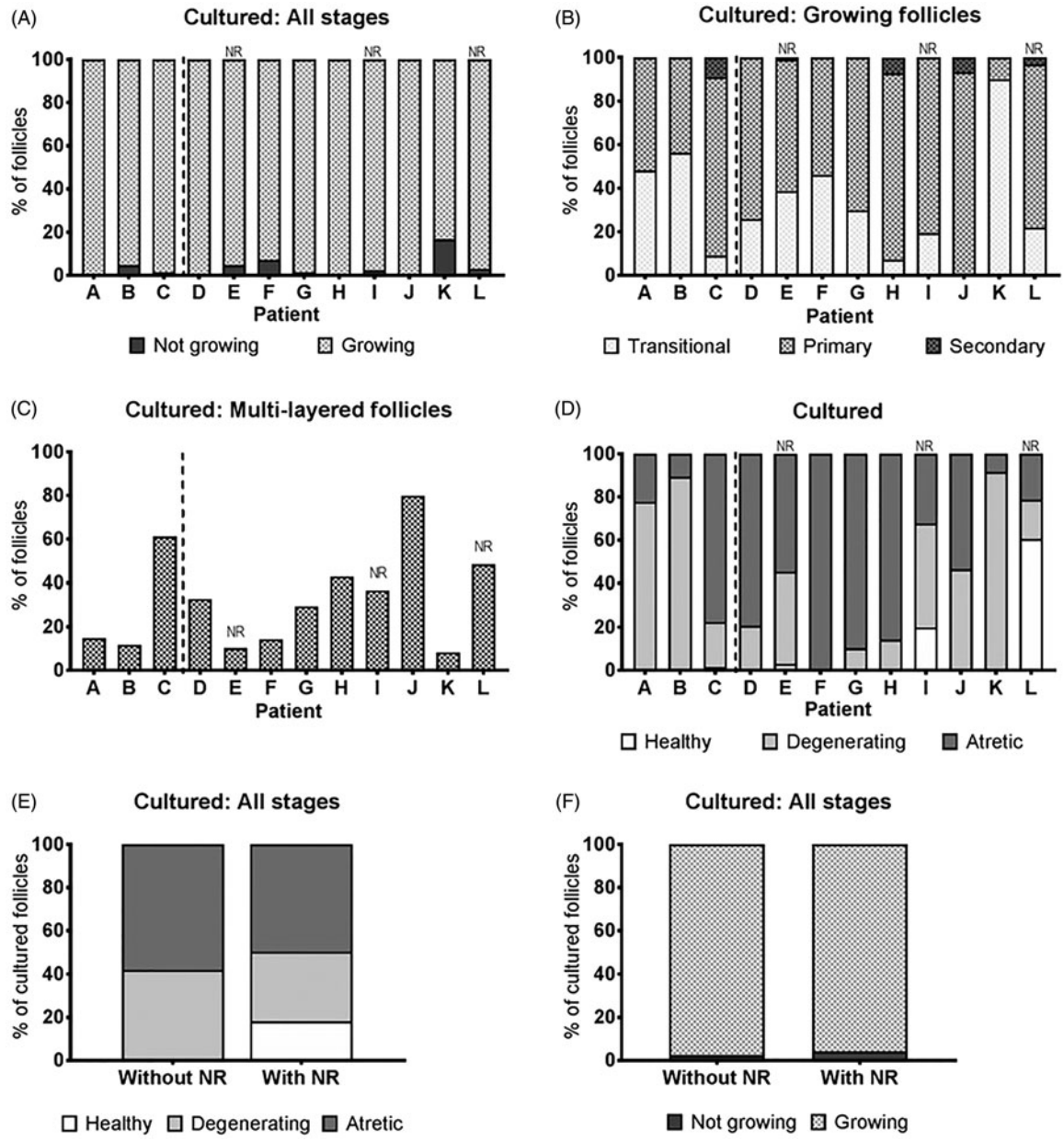


Figure 6. Variation between patients in percentage of growing and healthy follicles and the effect of tissue selection using neutral red in cultured cryopreserved ovarian tissue from patients diagnosed with malignant disease or blood disorder. There was no variation in the overall number of growing follicles in cultured cryopreserved ovarian cortical tissue (A) ($p = 0.289$, Fisher's Exact Test), however, within the growing population of follicles in cultured tissue (B), the relative number of transitional, primary and secondary follicles varied between patients ($p < 0.001$, Fisher's Exact Test), as did the percentage of multi-layered follicles, multilaminar follicles with more than one layer of granulosa cells (C). There was significant variation in the number of healthy, degenerating and atretic follicles between patients (D) ($p < 0.001$, Fisher's Exact Test). Tissue selection using neutral red had a significant impact on the proportion of healthy follicles present (E) ($p < 0.001$, OR = 108.9, 95% CI (12.3–962.8), logistic regression adjusted for tissue volume with patient as a random effect) but did not impact the number of growing follicles (F) ($p > 0.05$, Fisher's Exact Test). NR: neutral red staining used to select tissue.

asymmetry on subsequent follicle growth and developmental competence of the enclosed oocytes, particularly since current follicle selection for further development would not differentiate between them and non-asymmetric follicles.

Follicle health declines over the course of culture and there is evidence to suggest that this decline is more marked in cryopreserved tissue (Hovatta, Silye, Abir, Krausz, & Winston, 1997). We assessed if tissue selection using NR-staining would lead to better follicle development after culture. When NR was not used, nearly all follicles showed some sign of degeneration after 6 d of culture which was unsurprising given that only 22% were healthy in the non-cultured tissue.

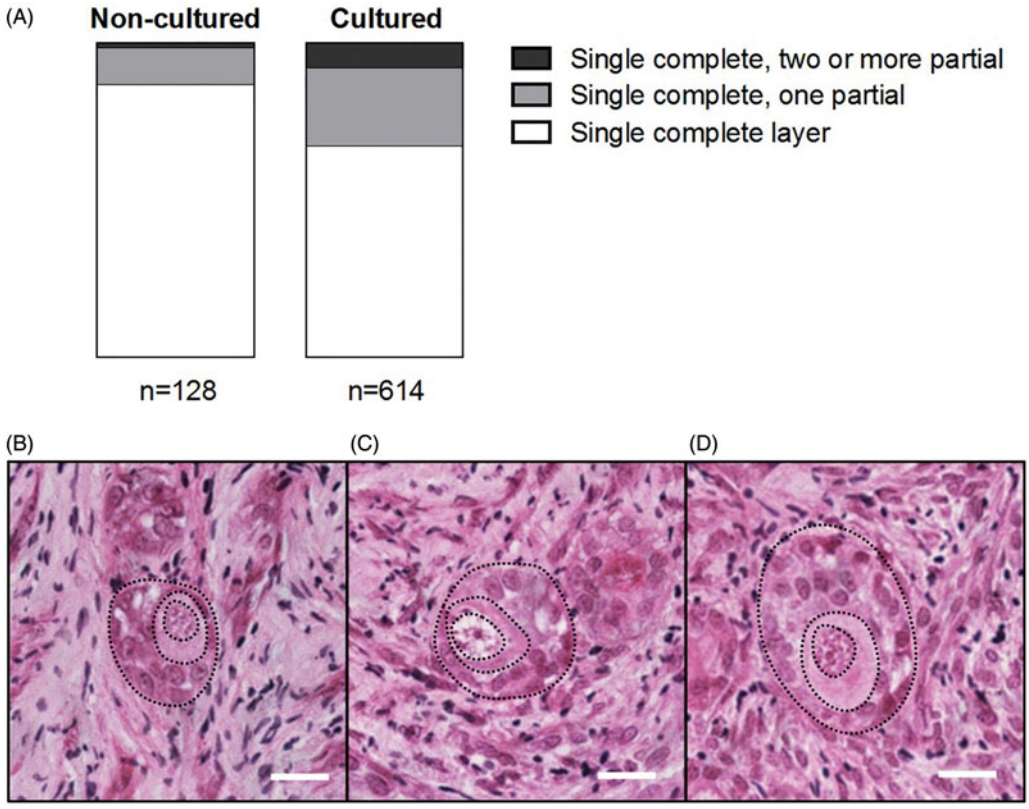


Figure 7. Asymmetric granulosa cell layers were more common in primary follicles in cultured cryopreserved tissue from patients diagnosed with malignant disease or blood disorder. Asymmetric primary follicles with variable numbers of partial granulosa cell layers were observed in non-cultured and cultured tissue. Primary follicles were significantly more likely to be asymmetric (those with a single complete layer of granulosa cells and two or more partial layers) with culture (A) (OR = 1117.3, 95% CI (250.2–4990.7), $p < 0.0001$, logistic regression adjusted for tissue volume with patient as a random effect). A follicle with a single complete granulosa cell layer and one partial layer is shown in image (B). Asymmetric primary follicles with a single complete granulosa cell layer and two or three partial layers are shown in images (C,D), respectively. Scale bar = 20 μm.

By contrast from 60% healthy follicles present in NR-selected non-cultured tissue, 18% of follicles retained a healthy morphology. These results are consistent with the 2.5-to-3-fold decrease in the proportion of healthy follicles reported by other groups culturing cryopreserved tissue over a similar timeframe, despite differences in culture systems (Asadi-Azarbaijani et al., 2016; Azarbaijani et al., 2015; Hovatta et al., 1997; Sanfilippo et al., 2013).

Although this analysis was based on a small volume of tissue per patient, an inverse correlation between age and follicle density was observed, confirming and extending that reported by Schmidt et al. (2003) with a sample of tissue from 21 women. The heterogeneity in follicle distribution within the human cortex is well described (Poirot et al., 2002; Qu, Godin, Nisolle, & Donnez, 2000; Schmidt et al., 2003), and has been cited as a strong reason that multiple pieces of ovarian cortex should be replaced upon re-implantation. It is possible that this heterogeneity extends to the response of follicles within a single ovary to the effects of cryopreservation, culture, or transplantation and the variation between patients reported in this study lends support to this idea.

In conclusion, *in vitro* development of eggs remains an exciting option for fertility restoration, but variation between patients may necessitate a more individual approach to downstream treatment options. We demonstrated here for the first time that there is variation between patients both in the health of non-cultured tissue post-cryopreservation as well as the health and development after 6 d in culture. The culture system described was effective in initiating follicle activation and growth in this tissue, but optimization is required to improve the survival of follicles across the culture period. Given the heterogeneity of tissue and clear variation in follicle health post-thaw in these patients, demonstrating that using the non-toxic viability marker NR in the existing culture workflow as described here to select tissue is an important advance in improving the efficiency and effectiveness of *in vitro* follicle development as an alternative method of fertility restoration.

Acknowledgements

The authors would like to thank all staff and the Oxford Cell and Tissue Biobank for their assistance in obtaining patient samples. Thanks go to Prof. Norah Spears for assistance in establishing culture techniques.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The study was supported by a joint scholarship to C.A.W from the Clarendon fund and NDWRH, and by an Oxford-MRC Doctoral Training Partnership (Oxford-MRC DTP) to B.D.B.

References

Allison, A., & Young, M. (1964). Uptake of dyes and drugs by living cells in culture. *Life Sciences*, *3*, 1407–1414. doi: 10.1016/0024-3205(64)90082-7.

Anderson, R.A., Wallace, W.H.B., & Telfer, E.E. (2017). Ovarian tissue cryopreservation for fertility preservation: Clinical and research perspectives. *Human Reproduction Open*, *2017*, hox001. doi: 10.1093/hropen/hox001.

Asadi-Azarbaijani, B., Sheikhi, M., Nurmio, M., Tinkanen, H., Juvonen, V., Dunkel, L., … Jahnukainen, K. (2016). Minimal residual disease of leukemia and the quality of cryopreserved human ovarian tissue *in vitro*. *Leukemia and Lymphoma*, *57*, 700–707. doi: 10.3109/10428194.2015.1065980.

Azarbaijani, B.A., Sheikhi, M., Oskam, I.C., Nurmio, M., Laine, T., Tinkanen, H., … Jahnukainen, K. (2015). Effect of previous chemotherapy on the quality of cryopreserved human ovarian tissue in vitro. *PLoS One*, *10*, e0133985. doi: 10.1371/journal.pone.0133985.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. doi: 10.18637/jss.v067.i01.

Borenfreund, E., & Puerner, J.A. (1985). Toxicity determined in vitro by morphological alterations and neutral red absorption. *Toxicology Letters*, *24*, 119–124. doi: 10.1016/0378-4274(85)90046-3.

Chambers, E., Gosden, R., Yap, C., & Picton, H. (2010). In situ identification of follicles in ovarian cortex as a tool for quantifying follicle density, viability and developmental potential in strategies to preserve female fertility. *Human Reproduction*, *25*, 2559–2568. doi: 10.1093/humrep/deq192.

De Vos, M., Smitz, J., & Woodruff, T.K. (2014). Fertility preservation in women with cancer. *The Lancet*, *384*, 1302–1310. doi: 10.1016/S0140-6736(14)60834-5.

Gougeon, A. (1986). Dynamics of follicular growth in the human: A model from preliminary results. *Human Reproduction*, *1*, 81–87. doi: 10.1093/oxfordjournals.humrep.a136365.

Hovatta, O., Silye, R., Abir, R., Krausz, T., & Winston, R. (1997). Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. *Human Reproduction*, *12*, 1032–1036. doi: 10.1093/humrep/12.5.1032.

Hovatta, O., Silye, R., Krausz, T., Abir, R., Margara, R., Trew, G., … Winston, R.M.L. (1996). Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. *Human Reproduction*, *11*, 1268–1272. doi: 10.1093/oxfordjournals.humrep.a019370.

Kristensen, S.G., Liu, Q., Mamsen, L.S., Greve, T., Pors, S.E., Bjørn, A.B., … Andersen, C.Y. (2018). A simple method to quantify follicle survival in cryopreserved human ovarian tissue. *Human Reproduction*, 33, 2276–2284. doi: 10.1093/humrep/dey318.

McLaughlin, M., Albertini, D.F., Wallace, W.H.B., Anderson, R.A., & Telfer, E.E. (2018). Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system. *MHR: Basic Science of Reproductive Medicine*, *24*, 135–142. doi: 10.1093/molehr/gay002.

McLaughlin, M., Kinnell, H.L., Anderson, R.A., & Telfer, E.E. (2014). Inhibition of phosphatase and tensin homologue (PTEN) in human ovary *in vitro* results in increased activation of primordial follicles but compromises development of growing follicles. *Molecular Human Reproduction*, *20*, 736–744. doi: 10.1093/molehr/gau037.

Nisolle, M., Casanas-Roux, F., Qu, J., Motta, P., & Donnez, J. (2000). Histologic and ultrastructural evaluation of fresh and frozen-thawed human ovarian xenografts in nude mice. *Fertility and Sterility*, *74*, 122–129. doi: 10.1016/S0015-0282(00)00548-3.

Poirot, C., Vacher-Lavenu, M.C., Helardot, P., Guibert, J., Brugières, L., & Jouannet, P. (2002). Human ovarian tissue cryopreservation: Indications and feasibility. *Human Reproduction*, *17*, 1447–1452. doi: 10.1093/humrep/17.6.1447.

Qu, J., Godin, P.A., Nisolle, M., & Donnez, J. (2000). Distribution and epidermal growth factor receptor expression of primordial follicles in human ovarian tissue before and after cryopreservation. *Human Reproduction*, *15*, 302–310. doi: 10.1093/humrep/15.2.302.

Sanfilippo, S., Canis, M., Romero, S., Sion, B., Déchelotte, P., Pouly, J.L., … Brugnon, F. (2013). Quality and functionality of human ovarian tissue after cryopreservation using an original slow freezing procedure. *Journal of Assisted Reproduction and Genetics*, *30*, 25–34. doi: 10.1007/s10815-012-9917-5.

Schmidt, K.L., Byskov, A.G., Nyboe Andersen, A., Muller, J., & Yding Andersen, C. (2003). Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. *Human Reproduction*, *18*, 1158–1164. doi: 10.1093/humrep/deg246.

Telfer, E.E., McLaughlin, M., Ding, C., & Thong, K.J. (2008). A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of active in. *Human Reproduction*, *23*, 1151–1158. doi: 10.1093/humrep/den070.

Xiao, S., Zhang, J., Romero, M.M., Smith, K.N., Shea, L.D., & Woodruff, T.K. (2015). *In vitro* follicle growth supports human oocyte meiotic maturation. *Scientific Reports*, *5*, 17323. doi: 10.1038/srep17323.

Analysing methods of cryopreserved human ovarian cortical strip culture to maximise follicle survival

B. D. Bjarkadottir[a#], C. A. Walker[a#], M Fatum[c], S. Lane[b] and S. A. Williams[a*]

[a]Nuffield Department of Women's and Reproductive Health, University of Oxford, Oxford, UK; [b]Department of Paediatric Oncology and Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford, UK; [c]Oxford Fertility, Institute of Reproductive Sciences, Oxford, UK.

#: equal contribution to work.

*Corresponding author:

Suzannah A. Williams

suzannah.williams@wrh.ox.ac.uk

Nuffield Department of Women's and Reproductive Health, University of Oxford

Level 3, Women's Centre, John Radcliffe Hospital, Oxford, United Kingdom, OX3 9DU

[Average journal in HumFert is around 2500 words according to journal guidelines]

[Word count (Main body): 2827 words]

Abstract [max 200]

In vitro follicle growth is a potential fertility preservation method for patients for whom current methods are contraindicated. Since many patients who may benefit from this treatment currently have cryopreserved ovarian tissue in storage, optimising *in vitro* follicle growth for cryopreserved-thawed tissue is critical. This study sought to determine the optimal culture method for cryopreserved human ovarian tissue by investigating the effect of culture medium (αMEM and McCoy's 5A), medium volume (1 mL on a membrane and 300 µL) and dish permeability on the health and development of follicles after six days of culture. A total of 5797 follicles from three post-pubertal patients (aged 21.3 ± 2.3 years) were analysed across six different culture conditions and non-cultured control. Differences in follicle morphology were evident with follicles cultured in low volume conditions having significantly greater odds of being graded as healthy compared to other conditions. Furthermore, culture in a low volume of αMEM resulted in the highest proportion of healthy primary and multilayer follicles (23.8% compared to 6.3-19.9% depending on condition). We therefore recommend culture of cryopreserved human ovarian tissue in a low volume of αMEM to best support follicle health and development.

Keywords: cryopreservation; ovarian tissue; organ culture; follicle; oocyte; human

Introduction

Advances in cancer treatment have led to increased survival rates, particularly among young children and adolescents. The increased number of young cancer survivors highlights the need for effective fertility preservation methods for these individuals. Current methods of female fertility preservation include cryopreservation of oocytes, embryos and ovarian tissue (Yasmin *et al.*, 2018). However, pre-pubertal girls are unable to undergo oocyte or embryo cryopreservation and for some of these patients, reimplantation of ovarian tissue is contraindicated due to the risk of reintroducing malignant cells. There is therefore a need to develop alternative fertility preservation methods for these patients.

One potential method of fertility preservation is *in vitro* follicle growth (IVFG) by culturing frozen ovarian tissue followed by *in vitro* maturation (IVM) of immature oocytes resulting in mature developmentally competent eggs. One of the limiting steps for IVFG is the generation of multilayer follicles from more immature follicles. Thus, optimisation of the initial step of ovarian tissue culture to facilitate the development of numerous multilayer follicles is critical for the success and subsequent clinical application of this method.

McLaughlin *et al* recently reported successful generation of metaphase II oocytes from cultured human ovarian tissue using a multi-step culture system (McLaughlin *et al.*, 2018). The first step involves culturing small fragments of fresh cortical tissue in a low volume of McCoy's 5A-based medium. In contrast, for culturing cryopreserved human ovarian cortical strips, many research groups use αMEM medium (Asadi-Azarbaijani *et al.*, 2016; Garor *et al.*, 2009; Huang *et al.*, 2008; Isachenko *et al.*, 2012; Lerer-Serfaty *et al.*, 2013; Ramezani *et al.*, 2017; Scott *et al.*, 2004). However, there has been no reported comparison between the two culture media to determine which is optimal for culture of

cryopreserved human ovarian tissue. In addition, it has been reported that increased oxygen availability by culturing fresh human ovarian tissue in gas-permeable culture plates had a positive impact on follicle health and development (Talevi *et al.*, 2018) and thus this is another variable that needs to be investigated. Since many patients who will need IVFG have already undergone ovarian tissue cryopreservation, it is important that IVFG techniques are established for both fresh and cryopreserved tissue samples.

This study aimed to determine the optimal culture method for cryopreserved human ovarian tissue by investigating the effect of culture medium, medium volume and gas permeable dishes on the health and development of follicles. We report here the effect each of these conditions on follicle progression and health after six days of culture, using statistical modelling to account for intra- and inter-patient variability.

Materials and methods

Ovarian tissue collection

The use of human tissue was approved by Health Research Authority South Central – Oxford B Research Ethics Committee (REC reference: 14/SC/0041). Cryopreserved ovarian tissue was obtained from the Oxford Cell and Tissue Biobank. Patient selection criteria included post-pubertal patients undergoing unilateral oophorectomy and subsequent ovarian tissue cryopreservation due to malignancy or blood disorder. Exclusion criteria included ovarian cancer and prior chemotherapy or radiation treatment. As part of the consent process, permission to use tissue in research had been obtained. Cortical strips were cryopreserved by the Oxford Cell and Tissue Biobank in 1.5 M ethylene glycol, 0.1 M sucrose and 10% serum substitute supplement in Leibovitz L-15 medium and stored in vapour phase liquid nitrogen.

Chemicals and consumables

Leibovitz L-15 medium (11415049), minimum essential medium alpha (αMEM) (22561021), McCoy's 5A (modified) HEPES buffered medium (22330021), L-glutamine (25030024) and ascorbic acid (10012011) were purchased from Thermo Fisher (Paisley, UK). Human serum albumin (AI653), ITS liquid media supplement (100x; I3145), Penicillin and streptomycin (P0781) sodium pyruvate (S8636), neutral red (N2889), sucrose (S7903), ethylene glycol (324558), Whatman Nucleopore membranes (WHA110414), Bouin's solution (HT10132), Gill no 2 haematoxylin (GHS232), Eosin Y solution (HT110332) and DPX mountant (06522) were purchased from Sigma Aldrich (Poole, UK). Recombinant human follicle stimulating hormone (FSH; Gonal-F; Z1540) was purchased from Merck Serono (Feltham, UK). Corning Costart tissue culture treated 24-well plates were purchased from Scientific Laboratory Supplies (Nottingham, UK). Lumox® 24-well plates were generously provided by Sarstedt (Nümbrecht, Germany).

Tissue thawing and cortical strip culture

Cortical strips were thawed and processed for culture as described in (Walker *et al.*, 2019). Briefly, following thawing in a water bath (30°C for 3 minutes), cortical strips were washed through thawing solutions for 5 minutes each at room temperature to remove cryoprotectants. The thawing solutions contained a reversed ethylene glycol gradient (1.0 M, 0.5 M and 0 M), 0.1 M sucrose and 3 mg/mL human serum albumin (HSA) in L-15 medium. Thawed strips were transferred to dissection medium [3 mg/mL HSA, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine and 2 mM sodium pyruvate in L-15 medium] and mechanically chopped using a McIlwain tissue chopper (Campden Instruments Ltd, UK), after which the fragments were further cut manually using scalpels and forceps into approximately 0.5 x 0.5 x 0.25 mm pieces. Tissue fragments were incubated in 25 µg/mL neutral red for 1 hour to visualise fragments with viable follicles

as described previously (Walker *et al.*, 2019).

Tissue pieces were distributed randomly and evenly between six different culture conditions, fragments where red staining was observed were allocated before non-stained fragments. A portion of tissue was fixed overnight in Bouin's solution as a non-cultured control. Three different plate conditions were tested: a polycarbonate membrane (13 mm diameter, 8 µm pore size) floating in 1 mL of medium in a conventional 24-well culture plate, 300 µL of medium in a conventional 24-well plate, and 1 mL of medium in a gas-permeable Lumox® 24-well plate (Figure 1). Two different media were compared for each plate condition (i.e. six experimental conditions in total): McCoy's 5A and αMEM, both supplemented with 1 mg/mL HSA, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, 10 µg/mL insulin, 5.5 µg/mL transferrin, 5 ng/mL selenium, 50 µg/mL ascorbic acid and 12.5 IU/L recombinant human FSH. Cortical tissue pieces were cultured for six days at 37°C under 5% CO_2 in air, with medium changes every other day (half the medium removed and fresh medium added). Following the culture period all tissue pieces (9-12 pieces per condition per patient) were fixed in Bouin's solution overnight before storage in 70% ethanol at 4°C.

Histological analysis

Fixed ovarian tissue was dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. The wax-embedded tissue was entirely serially sectioned at 5 µm, mounted on glass slides and stained with haematoxylin and eosin.

Follicles were staged as previously described (Gougeon, 1996; Walker *et al.*, 2019) as primordial (single layer of flattened pre-granulosa cells), transitional (single layer with at least one cuboidal granulosa cell), primary (complete layer of cuboidal granulosa cells) and multilayer (at least one complete layer of granulosa cells plus one or more partial or complete layers). Follicles were graded according to health based on the

presence of pyknotic granulosa cells or oocyte and shrinkage of the ooplasm as previously described (Walker *et al.*, 2019). Healthy follicles were defined as having a non-pyknotic non-shrunken oocyte with non-pyknotic granulosa cells, degenerating follicles had one of the above factors, while follicles were classified as atretic if they had both an oocyte with a pyknotic nucleus and pyknotic granulosa cells. Every tissue section was analysed and each follicle was followed through neighbouring sections to avoid double counting. Only follicles with a visible nucleolus or a clearly defined nuclear membrane were assessed. Follicles were analysed by two independent researchers with at least 10% overlap to ensure consistency in staging and health grading.

The area of every 12th tissue section was measured using ImageJ 1.46r (National Institutes of Health, USA; Rueden *et al.*, 2017; Schneider *et al.*, 2012). Average area measurements were used to calculate the volume of each tissue piece. Follicle density was determined by dividing the total number of follicles counted in a tissue sample by the tissue volume.

Statistical analysis and modelling

All statistical analyses were performed using R statistical software, version 3.5.0. A generalised linear mixed model following a negative binomial distribution (glmmPQL; Venables and Ripley, 2002) was used to determine the effect of culture condition on follicle development, adjusted for patient and tissue volume. A proportional odds model (clmm2; Christensen, 2015) was used to determine whether follicle health was affected by culture condition, again adjusting for tissue volume and patient. Data are presented as mean (±SEM), combined proportions (%) from all patients or as odds ratios (OR) with 95% confidence intervals (CI), unless otherwise stated, and statistical significance was defined as $p < 0.05$.

Results

Patient characteristics

Samples from three post-pubertal patients (aged 17-25 years, mean 21.3 ± 2.3 years) were used in this study. Table 1 shows patient age, diagnosis and non-cultured follicle density. The total number of follicles analysed from all three patients across all conditions was 5797. There was great variation in follicle density in both cultured and non-cultured tissue between patients, ranging from 20.4 ± 5.5 to 431.9 ± 23.8 follicles/mm^3 (Table 1). This, coupled with our group's previous work (Walker *et al.*, 2019), highlighted the need for statistical modelling to account for this intra-patient variability.

Follicle development was predominantly unaffected by culture condition

Follicles were classified based on histology as primordial, transitional, primary or multilayer. Follicles grew during the culture period, with the majority of follicles being classified as primordial (70.4%) or transitional (27.2%) in non-cultured tissue, whereas after six days of culture 39.4-79.7% of follicles were at the primary or multilayer stages, depending on the culture condition (Figure 2).Tissue cultured in a low medium volume contained the highest proportion of multilayer follicles, 26.8% for McCoy's (ML) and 28.5% for αMEM (AL). Follicle development was compared across the different culture conditions, with all conditions being compared to culture in a high volume of McCoy's 5A medium on a polycarbonate membrane (MM) as this was our group's established culture method based on Lopes *et al.*, 2019 prior to this study (Walker *et al.*, 2019; Figure 3). There were significantly fewer transitional follicles in tissue cultured in a low volume of McCoy's 5A medium (ML) compared to membrane culture (MM; 7.8 ± 6.0 follicles/mm^3 versus 28.2 ± 20.8 follicles/mm^3 respectively, $p<0.05$, Figure 3B). There was no difference between the conditions for follicles at other stages.

Culture in low volume conditions improved follicle health

Follicles were classified as healthy, degenerating or atretic based on morphology. Non-cultured tissue contained mostly healthy follicles at all stages, however after six days of culture the proportion of degenerating or atretic follicles had increased considerably for all culture conditions (Figure 4). A proportional odds model was used to determine whether a follicle was more likely to be healthy compared to degenerating or atretic after six days in a particular culture condition (Figure 5). Compared to MM, tissue cultured in low volume conditions (McCoy's, ML or αMEM, AL) or permeable dish conditions (MP or AP) had greater odds of healthy multilayer follicles being observed compared to degenerating or atretic (Figure 5D). The most marked difference in the health of multilayer follicles was seen in the low volume conditions, particularly AL where the odds of observing a healthy follicle were 5 times greater than MM (OR=0.2; 95% CI 0.13-0.32 $p<0.001$). Tissue cultured in ML had 2.6 times greater odds of multi-layered follicles being classified as "healthy" follicles versus "degenerating" or "atretic" compared to MM (OR=0.38; 95% CI 0.23-0.64; $p<0.001$).

As culture in AL resulted in healthier follicles of all stages compared to MM, AL was set as the baseline level of comparison in the proportional odds model, to ascertain whether medium affected follicle health within the low volume condition. Compared to AL, multilayer follicles cultured in ML had 1.9 times greater odds of being classified as degenerating or atretic than healthy (OR=1.92; 95% CI 1.24-2.97; $p<0.001$) showing that AL yielded superior follicle health compared to the other conditions tested. When the proportion of healthy primary or multilayer follicles was compared between culture conditions, we observed that culture in AL yielded over 20% healthy primary or multilayer follicles, more than all other culture conditions (Figure 6).

Discussion

Here we report that culture medium and volume significantly impacts the health of follicles generated from six-day *in vitro* culture of cryopreserved human ovarian tissue. We found that culture in a low volume of αMEM resulted in healthier follicles at all stages of development compared to the same volume of McCoy's 5A medium, and a higher volume of both base media in conventional plates with a polycarbonate membrane and in gas-permeable plates. We used statistical modelling to account for inter- and intra-patient variability.

Our results demonstrate that culture in a low medium volume led to improved follicle health compared to an approximately three-fold higher medium volume with a polycarbonate membrane. One possible reason is that in the low volume conditions, there is likely to be a greater concentrations of paracrine factors released by follicles or their surrounding cells which may have contributed to decreased follicle atresia. Oxygen availability is also a contributing factor to follicle health (Morimoto *et al.*, 2007; Talevi *et al.*, 2018). While three different plate conditions were compared in the current study (polycarbonate membrane, low volume and gas-permeable dish), all provided equal access to the air as the ovarian tissue was located close to the medium-gas interface (Figure 1). By culturing cryopreserved ovarian tissue in AL we observed a high level of follicle progression, with 26.8% multilayer follicles out of 810 follicles from three patients, with 34.0% healthy follicles. Talevi *et al.,* (2018) reported that culture of fresh human ovarian tissue in 5 mL of αMEM medium (column height 1.4 mm) in a gas-permeable petri dish led to improved follicle development and health, reporting that out of 287 follicles from six patients, 19.5% were multilayer and 41.7% healthy after six days of culture. This culture method is similar to that of AL described in the current study (AL column height 1.6 mm), however the medium volume used by Talevi *et al.* was 15 times greater. Taking into account the beneficial effects of low volume conditions, culturing

tissue in a low volume of αMEM in gas-permeable plates may lead to even greater follicle health.

Culture in a low medium volume (ML and AL) yielded the highest proportion of healthy primary and multilayer follicles out of the six conditions tested, with AL resulting in superior follicle health at all stages. There is currently only one report of successful generation of mature human oocytes grown from ovarian tissue (McLaughlin *et al.*, 2018) and this involved culture of fresh ovarian tissue using a four-step culture system; the first of which was similar to ML as described in the current study. This first culture step is the most limiting since it determines the number of follicles available for subsequent culture. McLaughlin *et al.* reported isolation of 87 multilayer follicles (100-150 μm diameter) from 160 cultured fragments and therefore improving the health and development of early growing follicles could therefore improve the yield of isolated follicles for IVFG. Studies describing culture of controlled-rate cryopreserved human ovarian tissue, as we have used in this study are limited, because the majority of studies use fresh ovarian tissue (Lopes *et al.*, 2019; McLaughlin *et al.*, 2018; Talevi *et al.*, 2018; Telfer *et al.*, 2008). Fresh and cryopreserved human ovarian tissue may have different requirements *in vitro*, as has been demonstrated using animal models (Castro *et al.*, 2014), highlighting the need to optimise ovarian tissue culture for both fresh and cryopreserved tissue. This is indeed critical since there are many patients who already have tissue cryopreserved and may require IVFG to generate eggs.

Follicles at all stages were more likely to be graded as healthy when cultured in a low volume of αMEM (AL) compared to the same volume of McCoy's 5A medium (ML). αMEM contains both sodium pyruvate and a physiological concentration of glucose (5.6 mM), whereas McCoy's 5A contains no pyruvate and three times higher concentration of glucose (16.7 mM) and therefore McCoy's 5A may provide a more suitable environment

for follicle metabolism. Previous studies have indeed demonstrated pyruvate to be the main energy source during early follicle development in mice (Harris *et al.*, 2007, 2009). Under normal conditions the oocyte is supplied with pyruvate by its supporting somatic cells however it has been demonstrated that pyruvate is taken up from the culture medium during *in vitro* follicle culture (Harris *et al.,* 2009). Therefore, αMEM may provide a more suitable energy source for early follicle metabolism compared to McCoy's, thereby better supporting follicle health although it is unknown at this time if cryopreservation has any effect on subsequent tissue requirements in culture.

In conclusion, we report that culture in a low volume of αMEM in conventional 24-well plates led to improved follicle health after six days of culture compared to other conditions tested. This highlights the need to further optimise culture systems for ovarian tissue culture, particularly culture of cryopreserved ovarian tissue. Improving the health and early development of follicles *in vitro* is a key aspect of developing culture systems capable of supporting follicle development from the earliest stages to result in mature fertilisable eggs. Once developed, these methods could offer a significant number of individuals a chance of achieving pregnancy following cancer treatment.

Acknowledgements

The authors would like to thank all staff and the Oxford Cell and Tissue Biobank for their assistance in obtaining patient samples, in addition to the patients themselves for donating tissue to research. Thanks go to Prof. Norah Spears for assistance in establishing culture techniques. The authors are also grateful to Sarstedt for providing the Lumox® culture plates.

Disclosure statement

The authors report no conflict of interest.

Funding

This work was supported by the Oxford Medical Research Council Doctoral Training Programme (Oxford MRC-DTP) grant awarded to B.D.B. (grant number MR/N013468/1) and by a joint scholarship to C.A.W. from the Clarendon fund and NDWRH.

References

Asadi-Azarbaijani B, Sheikhi M, Nurmio M, Tinkanen H, Juvonen V, Dunkel L, Hovatta O, Oskam IC, Jahnukainen K. Minimal residual disease of leukemia and the quality of cryopreserved human ovarian tissue in vitro. *Leuk Lymphoma* 2016;**57**:700–707.

Castro SV, Carvalho AA, Silva CMG, Santos FW, Campello CC, de Figueiredo JR, Rodrigues APR. Frozen and Fresh Ovarian Tissue Require Different Culture Media to Promote in Vitro Development of Bovine Preantral Follicles . *Biopreserv Biobank* 2014;**12**:317–324.

Christensen RHB. ordinal-Regression Models for Ordinal Data. R package version 2015.6-28. *See http//www cran r-project org/package= ordinal* 2015.

Garor R, Abir R, Erman A, Felz C, Nitke S, Fisch B. Effects of basic fibroblast growth factor on in vitro development of human ovarian primordial follicles. *Fertil Steril* 2009;**91**:1967–1975.

Gougeon A. Regulation of Ovarian Follicular Development in Primates: Facts and Hypotheses. *Endocr Rev* 1996;**17**:121–155.

Harris SE, Adriaens I, Leese HJ, Gosden RG, Picton HM. Carbohydrate metabolism by murine ovarian follicles and oocytes grown in vitro. *Reproduction* 2007;**134**:415–424.

Harris SE, Leese HJ, Gosden RG, Picton HM. Pyruvate and oxygen consumption

throughout the growth and development of murine oocytes. *Mol Reprod Dev* 2009;**76**:231–238.

Huang L, Mo Y, Wang W, Li Y, Zhang Q, Yang D. Cryopreservation of human ovarian tissue by solid-surface vitrification. *Eur J Obstet Gynecol Reprod Biol* 2008;**139**:193–198.

Isachenko V, Mallmann P, Petrunkina AM, Rahimi G, Nawroth F, Hancke K, Felberbaum R, Genze F, Damjanoski I, Isachenko E. Comparison of in vitro- and chorioallantoic membrane (CAM)-culture systems for cryopreserved medulla-contained human ovarian tissue. *PLoS One* 2012;**7**:1–9.

Lerer-Serfaty G, Samara N, Fisch B, Shachar M, Kossover O, Seliktar D, Ben-Haroush A, Abir R. Attempted application of bioengineered/biosynthetic supporting matrices with phosphatidylinositol-trisphosphate-enhancing substances to organ culture of human primordial follicles. *J Assist Reprod Genet* 2013;**30**:1279–1288.

Lopes F, Liu J, Morgan S, Matthews R, Nevin L, Anderson RA, Spears N. Single and combined effects of cisplatin and doxorubicin on the human and mouse ovary in vitro. 2019:1–45.

McLaughlin M, Albertini DF, Wallace WHB, Anderson RA, Telfer EE. Metaphase II oocytes from human unilaminar follicles grown in a multi-step culture system. *Mol Hum Reprod* 2018:1–8.

Morimoto Y, Oku Y, Sonoda M, Haruki A, Ito K, Hashimoto S, Fukuda A. High oxygen atmosphere improves human follicle development in organ cultures of ovarian cortical tissues in vitro. *Hum Reprod* 2007;**22**:3170–3177.

Ramezani M, Salehnia M, Jafarabadi M. Short term culture of vitrified human ovarian cortical tissue to assess the cryopreservation outcome: Molecular and morphological analysis. *J Reprod Infertil* 2017;**18**:162–171.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 2017;**18**:1–26.

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;**9**:671–675.

Scott JE, Carlsson IB, Bavister BD, Hovatta O. Human ovarian tissue cultures: Extracellular matrix composition, coating density and tissue dimensions. *Reprod Biomed Online* 2004;**9**:287–293. Available at: http://dx.doi.org/10.1016/S1472-6483(10)62143-8.

Talevi R, Sudhakaran S, Barbato V, Merolla A, Braun S, Nardo M Di, Costanzo V, Ferraro R, Iannantuoni N, Catapano G, *et al.* Is oxygen availability a limiting factor for in vitro folliculogenesis? *PLoS One* 2018;**13**:e0192501.

Telfer EE, McLaughlin M, Ding C, Thong KJ. A two-step serum-free culture system supports development of human oocytes from primordial follicles in the presence of activin. *Hum Reprod* 2008;**23**:1151–1158.

Venables WN, Ripley BD. *Modern Applied Statistics with S*. Fourth. Springer, 2002.

Walker CA, Bjarkadottir BD, Fatum M, Lane S, Williams SA. Variation in follicle health and development in cultured cryopreserved ovarian cortical tissue: a study of ovarian tissue from patients undergoing fertility preservation. *Hum Fertil* 2019;**0**:1–11.

Yasmin E, Balachandren N, Davies MC, Jones GL, Lane S, Mathur R, Webber L, Anderson RA. Fertility preservation for medical reasons in girls and women: British fertility society policy and practice guideline. *Hum Fertil* 2018;**21**:3–26.

Legends

Table 1. Patient characteristics and non-cultured follicle density.

Figure 1. Overview of culture conditions. Cryopreserved human cortical strips were thawed and processed for culture as described in the methods section. Three plate conditions were tested along with two culture media, resulting in a total of six culture conditions. **(A)** Polycarbonate membrane floating in a high volume (1 mL) of McCoy's 5A (MM; Ai) or αMEM (AM; Aii) medium in a conventional 24-well plate. **(B)** Low volume (300 µL) of McCoy's 5A (ML; Bi) or αMEM (AL; Bii) medium in a conventional 24-well plate; **(C)** High volume (1 mL) of McCoy's 5A (MP; Ci) or αMEM (AP; Cii) medium in a gas-permeable Lumox® 24-well plate.

Figure 2. Follicle development after six-day culture of cryopreserved human ovarian tissue. Follicles were staged as primordial (single layer of flattened pre-granulosa cells), transitional (single layer with at least one cuboidal granulosa cell), primary (single layer of cuboidal granulosa cells) and multilayer (at least one complete layer of granulosa cells plus one or more partial or complete layers). Follicle development was observed across all culture conditions compared to non-cultured control (D0, separated by dashed line), with a high proportion of primary and multilayer follicles being observed in cultured samples. The numbers across the top of the columns represent the number of follicles analysed in each group, combined from three patients. D0: non-cultured control; MM: McCoy's high volume with membrane, AM: αMEM high volume with membrane; ML: McCoy's low volume; AL: αMEM low volume, MP: McCoy's high volume, gas permeable plate; AP: αMEM high volume, gas-permeable plate.

Figure 3. Follicle development is largely unaffected by culture condition. Follicles were classified as primordial, transitional, primary or multilayer based on histology in non-cultured tissue and tissue cultured for six days in different conditions. Transitional follicle density was significantly lower in ML compared to MM ($p < 0.05$). There was no difference in the density of primordial, primary or multilayer follicles between the different conditions tested and MM. Data was analysed using a generalised linear mixed model. * $p < 0.05$. D0: non-cultured control; MM: McCoy's high volume, with

membrane AM: αMEM high volume with membrane; ML McCoy's low volume; AL: αMEM low volume, MP: McCoy's high volume, gas permeable plate; AP: αMEM high volume, gas-permeable plate.

Figure 4. Follicle health after six-day culture of cryopreserved human ovarian tissue. Follicles were classified as healthy (no morphological evidence of degeneration), degenerating (presence of either a pyknotic oocyte, pyknotic granulosa cells or shrunken ooplasm), and atretic (presence of both pyknotic oocyte and pyknotic granulosa cells). The proportion of atretic follicles was increased in cultured tissue compared to non-cultured control. The numbers across the top of the columns represent the number of follicles analysed in each group, combined from three patients. D0: non-cultured control; MM: McCoy's high volume with membrane, AM: αMEM high volume with membrane; ML McCoy's low volume; AL: αMEM low volume, MP: McCoy's high volume, gas permeable plate; AP: αMEM high volume, gas-permeable plate.

Figure 5. Effect of culture condition on follicle health. A proportional odds ratio model was used to determine whether any of the conditions tested was more likely to lead to healthier follicles at each stage compared to MM. Data is represented as odds ratio with upper and lower confidence intervals (displayed as error bars). Odds ratio < 1 indicates decreased odds of a follicle being degenerating or atretic compared to MM. Odds ratio > 1 indicates increased odds of a follicle being degenerating or atretic compared to MM. Those conditions where confidence intervals do not cross 1 (black lines) were significantly less likely to have degenerating or atretic follicles compared to the baseline (MM, $p<0.05$). MM: McCoy's high volume with membrane, AH: αMEM high volume with membrane; ML McCoy's low volume; AL: αMEM low volume, MP: McCoy's high volume, gas permeable plate; AP: αMEM high volume, gas-permeable plate.

Figure 6. Culture in low volume conditions yields healthier growing follicles. The proportion of healthy primary (dotted) and multilayer (checkerboard) follicles were compared across culture conditions. Culture in low volume conditions resulted in the highest proportion of healthy primary and multilayer follicles. Values represent combined values from three post-pubertal patients. The numbers across the top of the

columns represent the number of follicles analysed in each group, combined from three patients. D0: non-cultured control; MM: McCoy's high volume with membrane, AM: αMEM high volume with membrane; ML McCoy's low volume; AL: αMEM low volume, MP: McCoy's high volume, gas permeable plate; AP: αMEM high volume, gas-permeable plate.

Table 1.

Patient	Age (years)	Diagnosis	Total follicles analysed	Non-cultured follicle density (follicles/mm^3)
1	25	Cervical cancer	303	20.4 ± 5.5
2	17	Ewing's sarcoma (left pelvis)	3198	431.9 ± 23.8
3	22	Atypical teratoid rhabdoid tumour	2295	127.7 ± 77.3

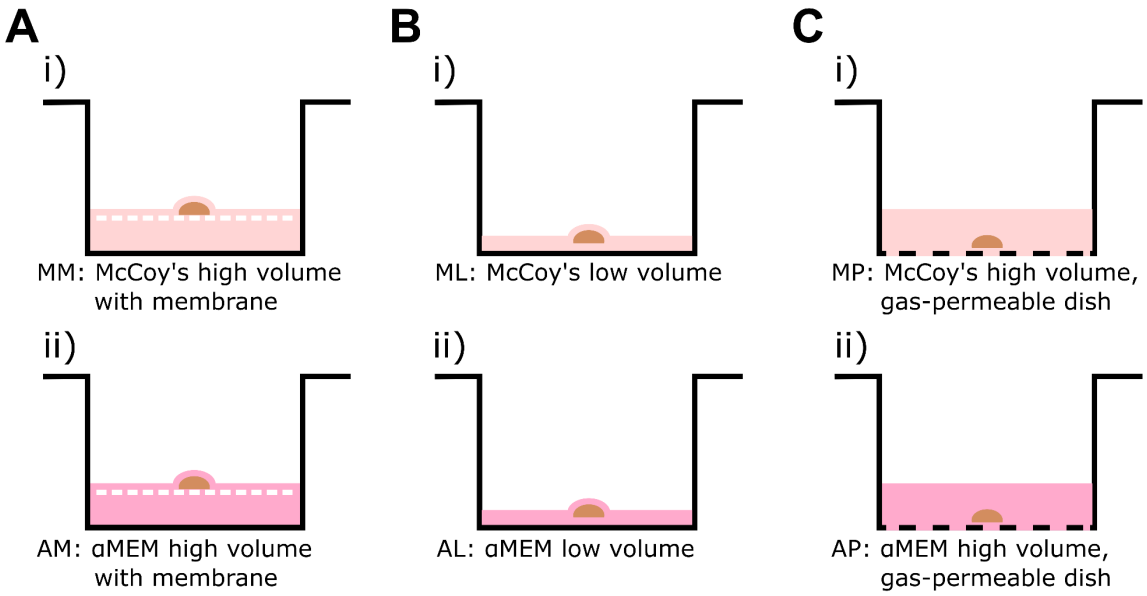


Figure 1

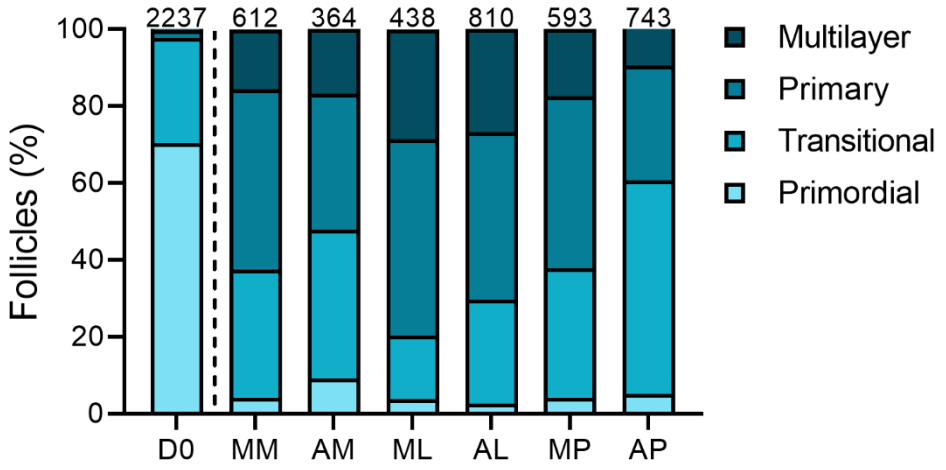


Figure 2

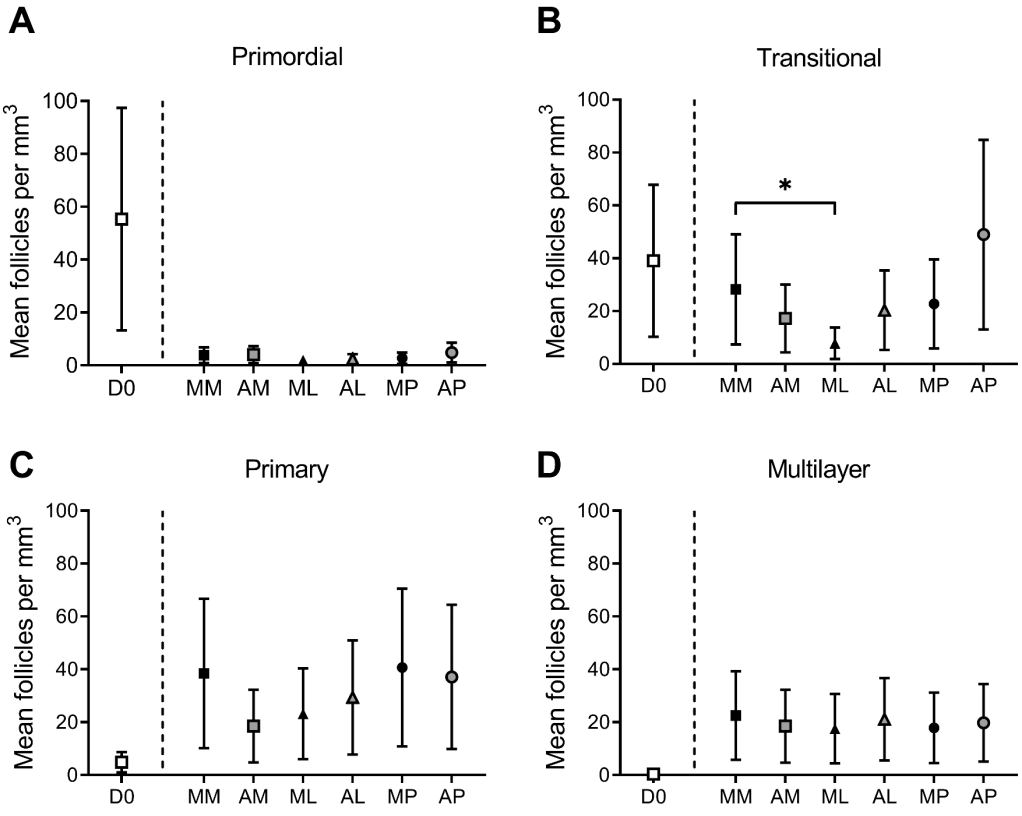


Figure 3

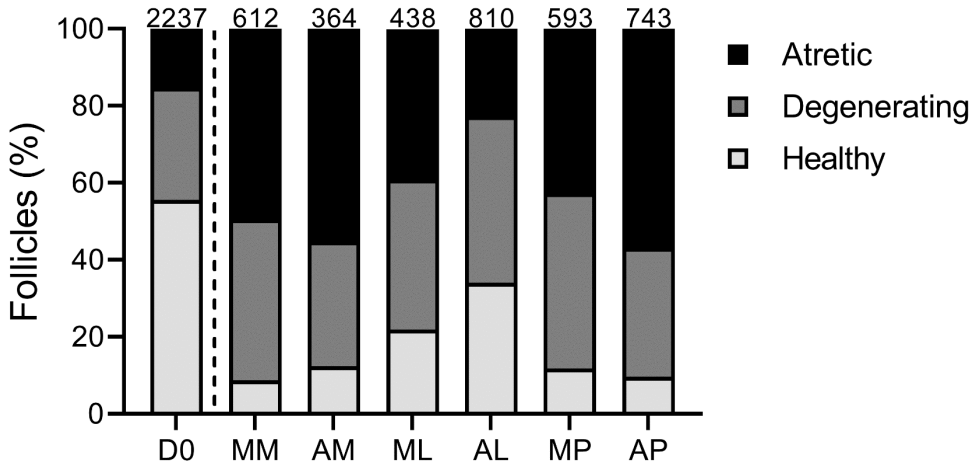


Figure 4

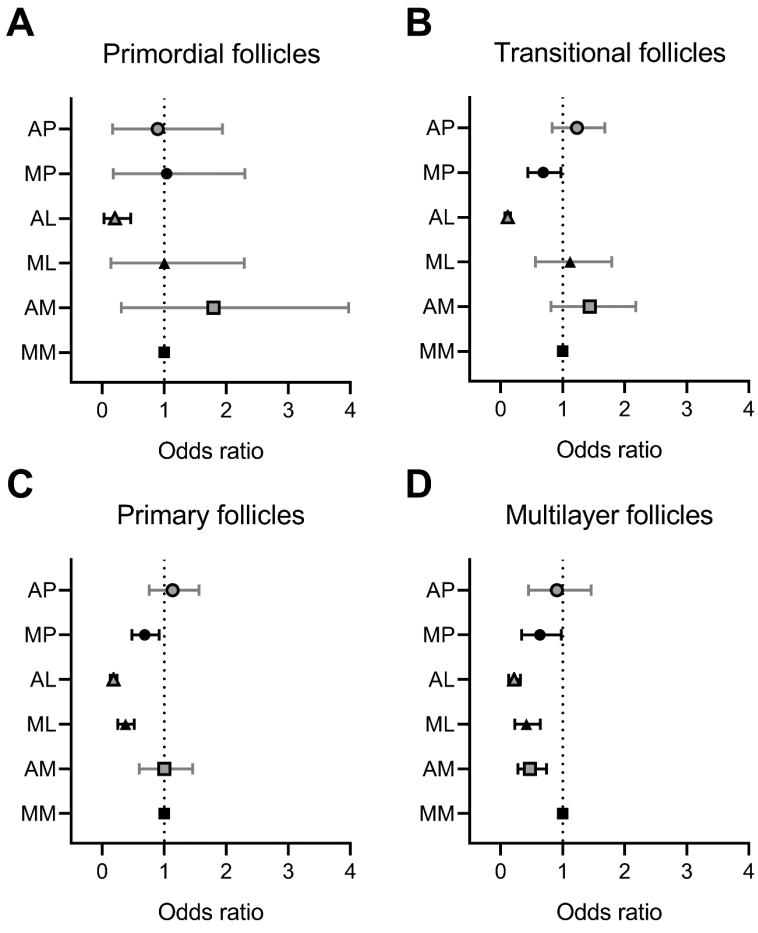


Figure 5

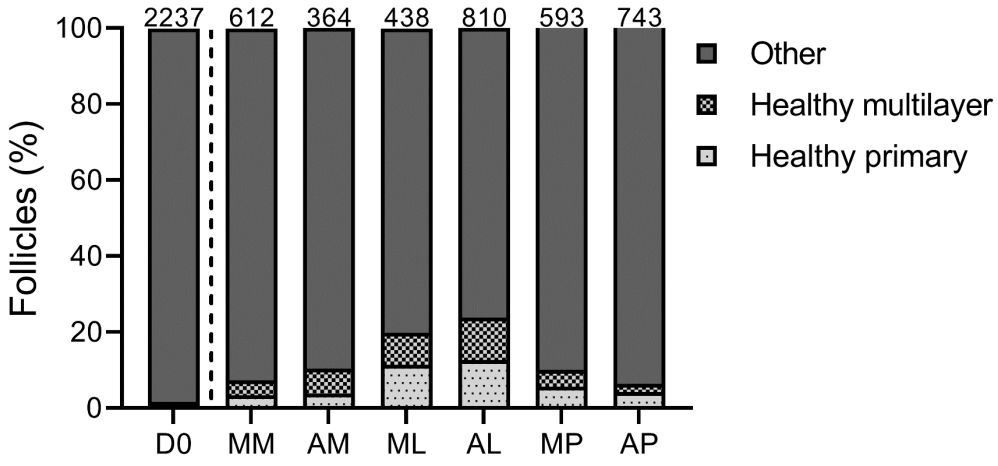

2237
612
364
438
810
593
743
100
80
60
40
20
0
Follicles (%)
D0
MM
AM
ML
AL
MP
AP
Other
Healthy multilayer
Healthy primary

Figure 6

FORM UPR16

Research Ethics Review Checklist

University of Portsmouth

Please complete and return the form to Research Section, Quality Management Division, Academic Registry, University House, with your thesis, prior to examination

Postgraduate Research Student (PGRS) Information			**Student ID:**	**956076**
Student Name:	**Muhammad Fatum**			
Department:	School of Pharmacy and Biomedical Sciences Faculty of Sciences	**First Supervisor:**	**Prof Graham Mills**	
Start Date: (or progression date for Prof Doc students)	**16 September 2019**			

Study Mode and Route:						
	Part-time	☒	MPhil	☐	Integrated Doctorate (NewRoute)	☐
	Full-time	☐	MD	☐	Prof Doc (PD)	☐
			PhD	☐		

Title of Thesis:	**Optimisation of fertility cryopreservation in females - from in vitro fertilization to in vitro maturation to in vitro culturing**
Thesis Word Count: (excluding ancillary data)	17205

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

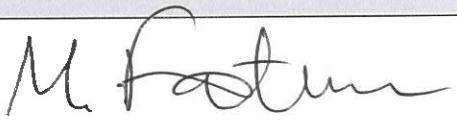
Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist:
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	**YES**

b) Have all contributions to knowledge been acknowledged?	**YES**
c) Have you complied with all agreements relating to intellectual property, publication and authorship?	**YES**
d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	**YES**
e) Does your research comply with all legal, ethical, and contractual requirements?	**YES**

*Delete as appropriate

Student Statement:	
I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)	
Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	All ethical permissions were obtained for each publication
Signed: *(Student)* 	**Date:17/03/2020**
If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain why this is so:	
Signed: *(Student)*	**Date:**